

Traditional herbs for modern medicine

Pioneering efforts by India's Central Drug Research Institute

Rakesh Maurya and C. M. Gupta

This article compares traditional therapeutic claims of important plant drugs with the investigative findings of modern science. It examines the therapeutic attributes of plants and their potential for use as modern herbal drugs. It demonstrates the process of technology transfer and commercialization by India's Central Drug Research Institute in the pharmaceutical area. Two case studies reveal that several factors have to work together to ensure success in technology transfer and commercialization in the biomedical area of a developing country.

Introduction

Traditional medicine refers to the ancient medical practice that existed in human societies before the application of modern science to health. The importance of traditional medicine (TM) as a source of primary health care was first officially recognized by the World Health Organization (WHO) in 1976 by globally addressing its Traditional Medicine Programme.

Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant-, animal- and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination, in maintenance of health and the treatment of diseases. The WHO estimates that about 80 per cent of world population relies on TM for primary health care.

Although modern medicine is widely spread, TM still exists in all countries. It is interesting to note that 25 per cent of modern medicines are derived from plants that were used traditionally. For example, the Chinese herbal remedy *Artemisia annua*, used in China for almost 2000 years, has been found to be effective against resistant malaria, and has created a breakthrough in preventing almost a million deaths annually, most of them of children, from severe malaria.

TM systems

The major systems of TM in South-East Asia are Ayurveda and Chinese TM. Ayurveda originated in India long back in the pre-Vedic period. The *Rigveda* and *Atharva-veda* (5000 BC), the earliest Indian documents have references on health and diseases.

Dr. Rakesh Maurya
Dr. C. M. Gupta

Central Drug Research Institute
Chattar Manzil Palace
Lucknow 226 001, India
Tel: (+91-522) 2612411 18
Fax: (+91-522) 2623405/2623938/
2629504
E-mail: drcmg@rediffmail.com

Ayurvedic texts like *Charaka Samhita* and *Sushruta Samhita* were documented about 1000 BC. Ayurveda, developed from the *Vedic* concept of life, became the important source of all systems of medical sciences. In course of time, it became a part of the culture and heritage of the people of the Indian subcontinent.

Ayurvedic medicinal preparations consist mainly of plant materials in the form of powders, semi-solid preparations, decoctions, elixirs and distillates. Many of them also contain inorganic chemical substances, minerals and animal products. Alcoholic extracts and alcoholic solutions of the ingredients, tinctures and elixirs are also frequently used in *Ayurvedic* medicine.

Over thousands of years, traditional Chinese medicine has developed a theoretical and practical approach to the treatment and prevention of diseases. The first documented source of Chinese medical theory, the *Huangdi Nei Jing* ("Inner Classic of the Yellow Emperor") was written between 300 and 100 BC. It describes the diagnosis and treatment of a huge range of disorders and gives advice about healthy lifestyles, exercise, and diet, which conforms remarkably well to current recommendations for the prevention of chronic diseases.

Some of the plants used in TM systems are presented in this review, particularly in the areas of fertility regulation, osteoporosis, diabetes, cancer, malaria and analgesics.

Fertility regulation

Fertility regulation with plants or plant preparations and medicaments has been mentioned in the ancient texts of indigenous systems of medicine of many countries. The use of plants as emmenagogues, abortifacients and as local contraceptives was well known to the ancient physicians of India.¹⁻⁴ Abortion was usually induced by the insertion of irritant twigs of plants or sticks coated with astringent plant juices into the cervix or by oral administration of plant decoctions to initiate uterine contractions for expulsion of the foetus.⁵ Emmenagogues, which

often increase menstrual flow in the non-gravid uterus, were extensively used to induce abortion. These agents include drastic purgatives and irritant volatile oils, which are believed to induce uterine contractions secondary to intestinal irritation and violent gastro-enteritis.

Even today, rural folks and aboriginal tribes all over the world, including India, are believed to use plant contraceptives to limit their family size.⁵ Traditional use of plants for fertility regulation in other countries, viz. China,⁶ Africa,⁷ Brazil,⁸ Haiti,⁹ Korea¹⁰ and Russia¹¹ is also evident from the available reviews. Farnsworth^{12,13} has published a comprehensive review on plants, including those used in traditional or folklore medicine, as sources of new fertility agents.

The powdered seeds of *Abrus precatorius* L. (Jequerity) are used as oral contraceptives by Central African tribes. A single dose of about 200 mg is said to be effective for 13 menstrual cycles. *Discorea composita* (Hemsl.) and other species of wild yam (*Discorea belophylla* Voigt.) were used by native American people for hundreds of years to block fertilization. *Dieffenbachia sequine* (Jacq.) or dumbcane was used to induce temporary sterility in females in Central America, South America and Cuba. In Mexico, the Caribbean and Central America, the root decoction of *Gossypium barbadense* (L.), is reportedly used to induce abortion. Tea made of the leaves of Solomon's Seal (*Polygonatum multiflorum*), drunk every day for a week, will produce permanent sterility. Hebrew women in Old Testament times who no longer wanted to bear children would drink a "Cup of Roots" to prevent childbearing.

Daucus carota L. seeds have been used as a contraceptive for over 2,000 years. They act both as a contraceptive and as an early-stage abortifacient. Women in the Appalachian Mountains, from Pennsylvania to North Carolina, stir a teaspoonful of the seeds into a glass of water and drink it every time after coitus. In several parts of India, women chew dry seeds every day to control their fertility.¹⁴ *Ruta graveolens* L. is one of the most an-

cient and effective contraceptive plants. Pregnant women were constantly warned against eating its leaves even in small amounts. The active ingredient is a volatile oil, whose very smell is said to induce abortion. It was usually used in an infusion to bring about a delayed menstruation, but it was also eaten daily in salads as a contraceptive. *Hedeoma pulegoides* L. has been known as a contraceptive and abortifacient to the ancient Greeks. It is taken in tepid water. According to Macer's Herbal, written in the 12th century, it should be taken in tepid wine.

Tanacetum vulgare L. was first mentioned as an emmenagogue in the medical writings of Saint Hildegard of Bingen, a twelfth century German Benedictine nun. Two or three teaspoons of the crushed dried leaves and flowers would be steeped in 8-12 ounces of hot water for about half an hour, then strained and drunk over a period of several hours. *Senecio aureus* L. has been used in folk and native American medicine for ages, as an emmenagogue. The dried and crushed flowers, stems and leaves were steeped in very hot water for an hour or so, and then drunk in small cupfuls for a day or two until menstruation began. *Artemisia absinthium* L. has been used as an antifertility agent since ancient times in Greece. It induces abortion. Other species, such as *A. vulgaris* (Mugwort) is also emmenagogic. A strong tea made from any species of *Artemisia* is used to promote menstruation. *Caulophyllum thalictroides* L., Blue Cohosh, has been used for centuries by native American women to induce menstrual flow.¹⁴

In India, Egypt,¹⁵ Korea,¹⁰ Jordan and Saudi Arabia¹⁶ women have taken the *Ricinus communis* L. beans to prevent pregnancy. In Algiers, women used to dip castor beans in the warm blood of a rabbit before ingesting the beans to prevent pregnancy.¹⁷

Ayurvedic physicians of India use *Rivea hypocrateriformis* Choisy to prevent fertility in women.¹⁸ Traditional physicians in and around Kotagiri village near Ootacamund use a mixture of powder roots of *Cassia occidentalis*

is Linn., *Derris brevipes* and *Justicia simplex* to control fertility.¹⁹ In Koraput district of Orissa, tribal women make pea-sized pills of white seeds *Abrus precatorius* Linn. with black pepper and take one every morning for 21 days on completion of menstruation.^{5, 20} In Mayurbhanj district, a decoction of *Achyranthes aspera* Linn.²⁰ is taken with honey for 5-7 days within a month of conception for abortion and *Hibiscus rosasinensis* Linn. Flower^{5, 20} is powdered with henna (*Lawsonia inermis* Linn.) root and is taken once a day for 3-7 days from the third day of periods to avoid conception. In Phulbani district, tribal women take a root powder of *Plumbago rosea* Linn.^{5, 20} with milk once a day for three days after cessation of menses to avoid conception, and also the root powder of *Vinca rosea* Linn. orally with black pepper to terminate pregnancy.¹ In Kurab district they take *Plumbago zeylanica* Linn. root powder thrice a day to terminate a pregnancy as old as three months.^{5, 20} Further, a three-month-old pregnancy is terminated by tribal women of Kalahandi district of the state of Orissa by taking a root decoction of *Stephania japonica* Miers.²¹ In Ganjam district women take a nearly centimetre long powdered root of *Gloriosa superba* Linn. with three seeds of black pepper, and take a single dose with milk to terminate pregnancy as late as 4 months.^{5, 20} In Kheri district of Uttar Pradesh, the root of *Poya (Basella alba)* Linn. is used as an anti-fertility agent after menstrual periods. In Mirzapur district, tribal women use the pseudostem of banana (*Musa sapientum* Linn.)²² for contraception. The pseudostem is crushed and mixed with jaggery and made into pills. Each pill is taken early in the morning for nine days after menstruation. In Netarhat plateau of Bihar, a decoction of the bark of *Celastrus paniculata* Willd. is prepared and given to pregnant women as an abortifacient.⁵ In Jhumritalaiya, a bark paste of *Ailanthus excelsa* Roxb. is given with water once a day for 2-3 days on an empty stomach, to terminate pregnancies as old as 2-3 months.⁵ Tribal women around Salem in Tamilnadu chew

leaves of *Bambusa arundinacea* Retz. Willd. in the morning and evening for 1-3 days to induce abortion of an early conception.²¹

Antiosteoporotic

Osteoporosis, which has been defined as a "state of low bone mass", is one of the major problems in our aging society. Osteoporosis results in bone fractures in older members of the population, especially in postmenopausal women. Osteoporosis and related fractures represent major public health concerns. It has been recognized as a global problem by the World Health Organization.²³ Pharmacological agents, that are used to manage osteoporosis, act by decreasing the rate of bone resorption, thereby slowing the rate of bone loss, or by promoting bone formation. A large variety of herbal drugs mentioned in TM systems to promote bone health and control of osteoporosis have been reported.²⁴

Various plants formulations have been screened and studied for the antiosteoporotic activity. *Glycine max*, commonly known as soybean, has been exhaustively studied for its antiosteoporotic activity. Its ethanol extract as well as its constituents, which are isoflavones and lignans, are very active antiosteoporotic agents, which has already been proved by the number of studies.

The effect of soybean ethanol extract on the activity of osteoblasts MC3T3-E1 cells has been studied. The extract increased survival ($P < 0.05$) and DNA synthesis ($P < 0.05$) of MC3T3-E1 cells at a concentration range of 0.01-0.1 g/l in a dose-dependent manner. Soy extract at a concentration of 0.05 g/l increased alkaline phosphatase (ALP) activity ($P < 0.05$) and collagen synthesis ($P < 0.05$) of MC3T3-E1 cells. The anti-estrogen tamoxifen eliminated the stimulation of MC3T3-E1 cells on proliferation, ALP activity and collagen synthesis by soy extract, indicating that the main action of soy extract on the osteoblastic MC3T3-E1 cells is similar to that of estrogen. Therefore, soy extract has a direct stimulatory effect on bone formation in cultured os-

teoblastic cells *in vitro*.²⁵ The total coumarins from the fruits of *Cnidium monnieri* increased bone mineral density of femur metaphysis of osteoporotic rats.²⁶

A herbomineral formulation OST-6 (osteocare) was evaluated for its inhibitory effect on the progress of bone loss-induced ovariectomy in rats. Each gram of OST-6 contains *Terminalia arjuna* (bark 250 mg), *Withania somnifera* (root 250 mg), *Commiphora mukul* (gum resin 280 mg) and *praval bhasma* (220 mg). Ovariectomized rats were administered with OST-6 at 250 and 500mg/kg b.wt. orally, daily for 90 days. On the 91st day, ovariectomized rats showed reduced bone mineral content and increased serum alkaline phosphatase levels, excretion of urinary calcium and pyridinium cross links levels. Histologically, sections revealed narrowed and disappeared trabeculae and widened medullary spaces. The total numbers of tartrate-resistant acid phosphatase (TRAP) positive cells were significantly increased both *in vivo* and *in vitro* methods.

OST-6 at a dose of 500mg/kg, significantly improved bone mineral contents and serum alkaline phosphatase levels; reduced the elevated urinary calcium and pyridinium cross links excretion and the number of TRAP positive cells; and reversed the histological features mentioned above.²⁷

A Chinese herbal medicine, *Hoehu ekki to (Bu-zong-yi-gi-tang)*, is composed of ten herbal medicines: a mixture consisting of 4.0 g of *Astragalus roots* (Ougi), 4.0 g of *Atractylodes lanceae* rhizome (*soujyutsa*), 4.0 g of *Panax ginseng* roots (*Ninjin*), 3.0 g of *Angelica* roots (*Touki*), 2.0 g of *Bupleuri* roots (*Saiko*) 2.0 g of *Zyzyphus* fruits (*Taisou*), 2.0 g of *Aurantia nobilis* pericarp (*Chinpi*), 1.5 g of *Glycyrrhiza* roots (*Kanzou*), 1.0 g of *Cimifugae* rhizome (*Shouma*) and 0.5 g of *Zingiberis* rhizome (*Shoukyou*) was prepared, from which 5.0 g 'Hoehu ekki to' was extracted with hot water, filtered, lyophilized, and stored at 4°C.

This medicine has been used for the treatment of oligospermia and as a post operative medication in Japan, on bone loss in rats treated with a go-

nadotropin-releasing hormone (GnRH) agonist. The administration of GnRH agonist reduced the bone mineral density in the whole femur to 91.0 per cent of that in the control group. However, administration of conjugated estrogens and *Hoehu ekki* to increased the serum concentrations of estradiol 16.8 and 5.3 times respectively compared with the concentration in the GnRH agonist treated group, resulting in the augmentation of the bone mineral density to 110.3 per cent and 106.2 per cent respectively. *Hoehu ekki* to enhances the reduced BMD and causes a slight elevation of the serum estradiol levels in the chemically castrated rats.²⁸ The methanol extract of stems of *Sambucus sieboldiana* inhibited bone resorption in organ culture. Oral administration of ethyl acetate fraction of methanolic extract (50, and 100mg/kg/d) to ovariectomized rats prevented the decrease of bone mineral density (BMD) of the lumbar (L₂₋₄) vertebra, indicating that ethyl acetate fraction is effective *in vivo*.²⁹ The ethanol extract of the plant *Cissus quadrangularis*, commonly known as *hadjod*, for its bone fracture healing property was evaluated for its antiosteoporotic activity in ovariectomized rat osteoporosis at two different dose levels - 500mg and 750mg per kg/day. Healthy female albino rats were divided into five groups of six animals each. The first group was sham operated and served as control. All the remaining groups were ovariectomized. Group 2 was fed with an equivalent volume of saline and served as ovariectomized control. Groups 3-5 were orally treated with raloxifen (5.4 mg/kg) and with the ethanol extract of *Cissus quadrangularis* (500 and 750 mg/kg) respectively.

The findings assessed on the basis of biomechanical, biochemical and histopathological parameters showed that the ethanol extract of the plant had a definite antiosteoporotic effect.³⁰ The preventive effect of a herbal formulation *Dae-Bo-Won-Chun* (DBWC), on the progress of bone loss induced by ovariectomy (OVX) was studied in rats. From light microscope analyses, porous or erosive appearances were observed on the surface

of the trabecular bone of the tibia in ovariectomized rats, whereas those of the same bone in sham-operated rats were composed of fine particles. The trabecular bone area and the trabecular thickness in ovariectomized rats, decreased by 50 per cent from those in sham-operated rats; these decreases were completely inhibited by the administration of DBWC at a concentration of 10mg/kg per day for 7 weeks. The mechanical strength of the neck of the femur was decreased by ovariectomy, and this was significantly suppressed by the administration of DBWC. Serum phosphorus, alkaline phosphatase and thyroxine levels in ovariectomized rats increased compared with those in sham operated rats, and the increases were completely inhibited by the administration of DBWC. These results strongly suggest that DBWC is effective in preventing the development of bone loss induced by ovariectomy in rats.³¹

Diabetes

Diabetes mellitus is a group of metabolic disorders in the endocrine system. The disease is found in all parts of the world and is rapidly increasing. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high content of blood glucose.

There are two type of diabetes, Type 1 and Type 2. Type 1, or insulin-dependent diabetes mellitus (IDDM), in which the body does not produce any insulin, most often occurs in children and young adults. People with Type 1 diabetes must take a daily insulin injection to stay alive. Type 1 diabetes accounts for 5-10 per cent of diabetes. Type 2, or noninsulin-dependent diabetes mellitus (NIDDM), in which the body does not produce enough, or does not properly use insulin, is the most common form of the disease, accounting for 90-95 per cent of diabetes. Type 2 diabetes is nearing epidemic proportions, due to an increased number of elderly people, and a greater prevalence of obesity and sedentary lifestyles. As a very common chronic disease, diabetes is becoming the third most lethal disease of mankind.³²

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Several plants and isolated compounds have been demonstrated to have anti-diabetic potential in NIDDM.^{33, 34} Some Indian plants that have been pharmacologically tested and shown to be of some value in diabetes mellitus are described below.

Aegle marmelose

An aqueous extract of the leaves (1 gm/kg for 30 days) significantly controlled blood glucose, urea, body weight, liver glycogen and serum cholesterol of alloxanized (60 mg/kg IV) rats as compared to controls and this effect was similar to insulin treatment.³⁵ The aqueous extract of leaves, when fed (1 gm/kg/ day) to STZ (45 mg/kg IV) diabetic rats for 2 weeks, decreased malate dehydrogenase levels (an enzyme known to increase in diabetes) in comparison to diabetic controls.³⁶ A further aqueous extract of leaves, administered orally for 28 days, also normalized STZ (45 mg/kg body weight) induced histo-pathological alterations in the pancreatic and kidney tissues of rats.³⁷

Coccinia indica is used in the Ayurvedic and Unani systems of medicine for the treatment of diabetes.³⁸ Oral administration of 500 mg/kg of *C. indica* leaves showed significant hypoglycemia in alloxan-diabetic dogs (45 mg/kg IV) and increased glucose tolerance in normal and diabetic dogs (OGTT and IVGT), respectively.³⁹ The oral feeding of an ethanol extract of the leaves (200 mg/kg) to 18 h fasted rats and STZ diabetic rats led to lowering of blood sugar by 23 and 27 per cent respectively, hepatic glucose-6-phosphatase by 19 and 32 per cent respectively, and hepatic fructose-1,6-bisphosphatase by 20 and 30 per cent respectively, as compared to controls.⁴⁰

The beneficial effects of leaves of *C. indica* have also been shown in a double-blind control trial enrolling 16 patients with uncontrolled maturity onset diabetes and 16 controls. Treatment was given for 6 weeks and 10

patients showed marked improvement in their glucose tolerance.⁴¹ In a clinical study (n-30), an oral administration of dried extract of *C. indica* (500 mg/kg for 6 weeks) significantly restored the raised activity of lipoprotein lipase and the levels of G-6 phosphatase and LDH, which otherwise increase in severe diabetics.⁴² As a single oral dose, the plant extract has been shown to exert a beneficial hypoglycemic effect in experimental animals and human diabetic subjects.⁴³

Feeding of water-soluble alkaloid fractions of alcohol extract (1 gm/kg) of *Coccinia indica* leaves to normal fasting guinea pigs showed hypoglycemic activity.⁴⁴ The oral administration of bark extract *Ficus bengalensis* showed a significant antihyperglycemic effect in STZ diabetic rats by raising serum insulin levels or inhibiting insulinase activity in the liver and kidney.⁴⁵ Oral administration of pelargonidin and leucopelargonidin derivative (100 mg/kg) isolated from the bark of *F. bengalensis* exerts significant hypoglycemic activity in normal and moderately alloxanized diabetic dogs (60 mg/kg IV injection).⁴⁶⁻⁴⁹

Gymnema sylvestre

The anti-hyperglycemic effect of dried leaf powder of *Gymnema sylvestre* was seen in alloxanized rabbits along with a decrease in the activity of gluconeogenic enzymes and reversal of pathological changes in the liver initiated during the hyperglycemic phase.⁵⁰ The oral feeding of powdered leaves of *G. sylvestre* (500 mg/rat) for 10 days significantly prevented intravenous beryllium nitrate-induced hyperglycemia in rats and normalized it in 4 days in comparison to 10 days in untreated rats.

However, no significant hypoglycemia was seen in normal rats who were daily fed with the leaves of *G. sylvestre* for 25 days.⁵¹ Oral administration of aqueous extracts of leaves of *G. sylvestre* (20 mg/day) for 20-60 days normalized blood sugar levels of STZ diabetic rats through β cell regeneration.^{52,53} Gymnemic acid isolated from *G. sylvestre*^{54,55} and triterpene glycosides isolated from plant inhibit-

ed glucose utilization in muscles.⁵⁶ Oral treatment of *G. sylvestre* leaves extract (400 mg) for 18-20 months plus conventional treatment showed beneficial effects in 22 NIDDM patients.⁵⁷

In a clinical observation, an aqueous decoction of *G. sylvestre* leaves (2 gm thrice daily) to 10 healthy persons (10 days) and 6 diabetic patients (15 days) significantly reduced the fasting and OGTT glucose level in all the groups except OGTT in healthy group.⁵⁸

Momordica charantia

This is the most popular herbal resource and is often used to treat diabetes.^{59,60} The anti-diabetic potential of *Momordica charantia* is well established in streptozocin- or alloxan-induced diabetic rats, mice and rabbit⁶¹⁻⁶³, genetically diabetic mice⁶⁴ and in humans with Type 2 diabetes.⁶⁵ Aqueous extracts of *M. charantia* improved OGTT after eight hours in normal mice and reduced hyperglycemia by 50 per cent after five hours in STZ diabetic mice.⁶⁶ Ethanol and acetone extract of *M. charantia* fruits (250 mg/kg dose PO) significantly lowered blood sugar in fasted as well as glucose loaded non-diabetic rats.^{67,68} A homogenized suspension of the vegetable pulp of *M. charantia* to 100 cases of moderate NIDDM subjects caused a significant reduction of postprandial serum glucose in 86 per cent cases and fasting glucose in 5 per cent cases.⁶⁹ The aqueous juice of *M. charantia* fruit exerted anti-hyperglycemic and antioxidant effect in the pancreas of STZ-diabetic mice.⁷⁰ Oral supplementation (0.5, 1 and 3 per cent) with freeze-dried powder of *M. charantia* for 14 days with and without 0.5 per cent cholesterol and 0.15 per cent bile acid in the diet resulted in a consistent decrease in serum glucose levels in normal rats only in the former group.⁷¹ The aqueous extract of unripe fruits of *M. charantia* has also been shown to partially stimulate insulin release from isolated β -cell of obese-hyperglycemic mice.⁷²

In a clinical trial, water-soluble extract of the fruits of *M. charantia* signifi-

cantly reduced blood glucose concentrations in the nine NIDDM diabetics on OGTT (50 gm). Fried *karela* fruits consumed as a daily supplement to the diet produced a small but significant improvement in glucose tolerance in diabetic subjects without any increase in serum insulin levels.⁷³ *Pterocarpus marsupium* studies conducted by various authors have shown hypoglycemic activity of the wood extract in different animal models.⁷⁴⁻⁷⁷ Pterostilbene isolated from *Pterocarpus marsupium* heart wood caused hypoglycemia in dogs (at the dose of 10 mg/kg IV). Higher doses (20, 30 and 50 mg/kg) caused initial hyperglycemia followed by hypoglycemia lasting for nearly five hours.⁷⁸⁻⁷⁹

This hypoglycemic effect was attributed to the presence of tannates in the extract. An orally administered water decoction of the bark (1 gm/100 gm body weight for 10 days) showed a hypoglycemic action in alloxanized diabetic rats.⁸⁰ A chronic administration of the infusion of wood powder for five days inhibited the rise in blood glucose level in rats after glucose loading.⁸¹

Epicatechin, a pure flavonoid, isolated from the ethanol extract of *P. marsupium* bark has also been shown to possess a significant anti-diabetic effect.⁸²⁻⁸⁵ Epicatechin has been shown to enhance insulin release and the conversion of proinsulin to insulin *in vitro*.⁸⁵ Marsupin and pterostilbene constituents significantly lowered blood glucose level in STZ diabetic rats and the effect was comparable to metformin.⁸⁶

An Indian, open, multicentric study assessing *vijayasar* in the treatment of newly-diagnosed or untreated NIDDM showed that the extract controlled fasting and post-prandial blood glucose levels in 67 out of 97 patients (69 per cent) by the twelfth week at doses of 2, 3 and 4 g in 73, 16 and 10 per cent patients respectively. Four patients were withdrawn from treatment due to excessively high post-prandial blood glucose levels. No significant change was observed in the mean levels of lipids. Other laboratory parameters remained stable during the designated treatment period of 12 weeks.⁸⁷

Syzigium cumini is widely distributed throughout India, and Indian folk medicine mentions its use for the treatment of DM.² Preliminary studies on *Syzigium cumini* seeds and leaves have shown a hypoglycemic effect.^{88, 89} Oral feeding of *S. cumini* (170, 240 and 510 mg/rat for 15 days) caused 50 per cent reduction in blood glucose of normal, fasted rats while chlorpropamide showed 52 per cent reduction.⁹⁰ In oral administration of the aqueous extract of seeds of *S. cumini* (2.5 and 5.0 gm/kg for 6 weeks), the hypoglycemic effect was most prominent at a dosage of 5.0 gm/kg.⁹¹ The daily administration of lyophilized powder of *S. cumini* (200 mg/kg) showed maximum reductions of 73.51, 55.62 and 48.81 as compared to their basal values in mild (plasma sugar-180 mg/dl, duration 21 days), moderate (plasma sugar 280 mg/dl, duration 120 days) and severe (plasma sugar-400 mg/dl, duration 60 days) diabetic rats.⁹²

Cancer

Cancer is the second leading cause of death in the world - one out of every four deaths is from cancer. The National Institute of Health (NIH) has estimated costs relating to cancer to be US\$ 156.7 billion. It is also important to note that 77 per cent of all cancers diagnosed people are 55 years of age or older.⁹³ With cancer taking such a toll on the population, both in lives and costs, the discovery of anti-cancer drugs has become very important.

Some of the most effective cancer treatments to date are natural products or compounds derived from natural products. The *Catharanthus roseus* (rosy periwinkle) was used in Cuba, The Philippines and South Africa for the treatment of inflammation, rheumatism and diabetes. The active principles, vinblastine and vincristine⁹⁴⁻⁹⁶ showed significant clinical anti-tumour activity against Hodgkins's and non-Hodgkins's lymphomas, acute lymphoblastic leukaemia, breast carcinoma Wilms' tumour, Ewing's sarcoma, neuroblastoma, hepatoblastoma and small cell lung cancer, thus achieving a prom-

inent role in modern cancer chemotherapy.^{97, 98}

The most significant anti-cancer drug discovered and developed is taxol, isolated in 1969 from the bark of the Pacific yew tree (*Taxus brevifolia*).⁹⁹ In early clinical trials in 1989, it was found to be effective in ovarian cancers and breast cancers, and since that time it has shown significant therapeutic benefits for other advanced malignancies. As a natural source of supply could not be relied upon, taxol and other taxoids have been produced by semi-synthetic conversions of a precursor compound, 10-deacetyl baccatin, found in renewable yew tree needles. The paclitaxel (taxol) story illustrates the great importance of conserving natural resources.

The resin podophyllin obtained from the *Podophyllum peltatum* root, is toxic and is used clinically to remove warts. The major constituent of the resin is the lignan, podophyllotoxin. Two semi-synthetic derivatives of podophyllotoxin, viz. etoposide and teniposide, were developed as chemically active agents.¹⁰⁰

Camptothecin is an anti-cancer drug, which was isolated from the Chinese ornamental tree, *Camptotheca acuminata*.^{101, 102} Camptothecin (as its sodium salt) was clinically approved by the NCI in the 1970s, but was later dropped because of severe bladder toxicity. Yet extensive research towards lead development or more effective derivatives was continued, and resulted in two effective derivatives, topotecan and irinotecan. Topotecan is used for the treatment of ovarian and small cell lung cancers, while irinotecan is used for the treatment of colorectal cancers.

Malaria

Malaria is undoubtedly one of the most serious and widespread human diseases. It is caused by a protozoan parasite (*Plasmodium* spp). *Anopheles* mosquitoes are the vectors that carry the malaria parasite, and the insecticide DDT was extensively used to kill mosquitoes in the 1950s. Thereafter,

due to resistance to DDT among *Anopheles* spp. and serious side-effects for human health and the environment, the use of DDT was stopped.

The isolation of the anti-malarial drug, quinine, from the bark of *Cinchona* species (*C. officinalis*), was reported in 1820 by Caventou and Pelletier. The bark had long been used by indigenous people of the Amazon region for the treatment of fevers, and was introduced into Europe to treat malaria.¹⁰³

Using the structure as a lead, chemists synthesized the anti-malarial drugs, chloroquine and mefloquine. Another plant used in the treatment of fevers for more than 2,000 years in traditional Chinese medicine is *Artemisia annua* (Quinchaosu), which yields the antimalarial agent artemisinin.^{104, 105} Its derivatives, artemether and artether, are currently in use against strains of malaria that are increasingly resistant to first line treatments (chloroquine and sulphadoxine in combination with pyrimethamine) and are considered to be the most effective anti-malarial agents on the market today.¹⁰⁶

Analgesics

In the ancient world, pain was one of the major ailments. The use of medicinal plants for the cure of pain possibly began with treatment by use of the crude extract of the poppy (*Papaver somniferum*), dating from around 6,000 years ago in Sumeria. Traditionally opium has been used as an astringent, an antispasmodic, an aphrodisiac, a diaphoretic, an expectorant, a hypnotic, a narcotic, and a sedative. But the effectiveness of the opium poppy as an analgesic is well known. Opium and its derivatives are used in the pharmaceutical industry as narcotic analgesics, hypnotics, and sedatives.

Morphine was isolated by Serturner in 1806, codeine by Robiquet in 1832 and the non-morphine alkaloid papaverine by Merck in 1848.¹⁰⁷ Ironically, heroin is a compound that has probably been most abused and has caused the most human anguish. A close relative, dextromethorphan is, in fact, used in most cough syrups today, but lacks the abuse potential of its chemical cousin.

Table 1: Products developed by Central Drug Research Institute

S. No.	Drugs	Source	Use	Licensee
1.	Isapent	Seed husk of <i>Plantago ovata</i>	Cervical dilatation (MPT)	Unichem Labs
2.	Gugulipid	Gum of <i>Commiphora mukul</i>	Hypolipidemic	Cipla Nicholas Piramal Ind. Ltd.
3.	ProMind	Whole plant of <i>Bacopa monniera</i>	Memory improvement	Lumen Marketing Co.
4.	Consap	Nuts of <i>Sapindus mukorossi</i>	Spermicidal	Hindustan Latex Ltd.

Central Drug Research Institute

The following case study analyzes a TM product for technology transfer to pharmaceuticals. The case study describes Central Drug Research Institute (CDRI) experience from the introduction of Ayurvedic knowledge to the successful launch of its own new drug in the market. The case study demonstrates the process of development and the commercialization of advanced technology in the country.

Drugs developed

The development of new drugs from natural products has been the avowed objective of the CDRI since its inception. The main thrust of the Institute's research programme has been on the discovery and development of drugs for reproductive health research (fertility regulation, breast cancer, prostate hyperplasia), tropical and infectious diseases (malaria, tuberculosis, leishmaniasis, filariasis) and aging-related disorders (diabetes and dyslipidemia, osteoporosis, thrombosis, stroke, dementia).

The major focus has been to obtain novel lead molecules from medicinal plants and to develop them as modern drugs, but the Institute is always open to alternative approaches to develop a standardized plant extract into a viable herbal preparation after judging its marketing potential as a 'herbal medicine'. Folkloric, traditional and indigenous plants are taken up for investigation.

All the plant samples are botanically authenticated and voucher specimens are preserved in the Botany Division. Repeat collections are made from the same location during the same season so as to minimize variation. Initially 95 per cent ethanolic ex-

tracts of the plants are prepared for biological screening. Wide ranges of biological test systems are reorganized so as to enable screening of the same plant extracts for a large variety of biological activities.

The Institute has acquired a computer-assisted robotic High Throughput Screening (HTS) system capable of screening 96 tests samples *in vitro* within a short time. So far, more than 6,000 samples of terrestrial plants have been screened. Bio-assay-linked chemical investigations on the identified active plants have been undertaken, resulting in the identification of some active constituents whose structures have been established.

All the herbal medicines follow the required development studies, including regulatory studies covering pharmacology, pharmacokinetics, toxicology and clinical trials, in order to ensure their safety and efficacy. As a result several herbal medicines have been developed and successfully commercialized (Table 1) and others are in various stages of development.

Case study: Gugulipid

Ayurvedic knowledge and Joint Research

India recently increased research on traditional Ayurvedic herbal medicines after observing that they are effective for conditions to which they have traditionally been applied. For example, the ancient Sanskrit text on Ayurveda, the *Sushruta Samhita*, noted that *Commiphora mukul* was useful in treating obesity and conditions equivalent to hyperlipidemia, or increased concentrations of cholesterol in the body. The plant has been used by Ayurvedic practitioners for at least 200 years and may have been in use since the writing of the *Sushruta*

Samhita more than 2,000 years ago.¹⁰⁸ In the study, the crude gum from *Commiphora mukul* significantly lowered serum cholesterol in rabbits with high cholesterol levels.¹⁰⁹

The plant substance also protected rabbits from cholesterol-induced atherosclerosis (hardening of the arteries). This finding led to pharmacological and toxicological studies that showed this herbal remedy to be effective in humans, with no adverse side effects.¹¹⁰ On the basis of available therapeutic information, gum guggul was taken up for detailed investigations for hypolipidaemic agents (in collaboration with Dr. Sukh Dev, National Chemical Laboratory, Pune).

While Dr. Sukh Dev identified the chemical components of the various fraction of gum guggul of *C. mukul*, Dr. S. Nityanand and co-workers at CDRI were engaged in evaluating the biological efficacy of different fractions and their toxicity profile. By treating the gum guggul with ethyl acetate, soluble and insoluble fractions were obtained. The activity was found in the ethyl acetate soluble fraction designated as guggulipid, while the insoluble fraction had no activity, instead it showed hepatotoxicity.¹¹¹⁻¹¹³

E and Z- Guggulsterones

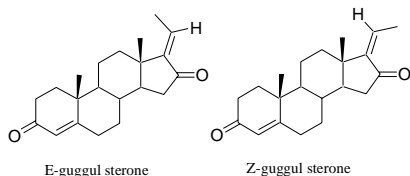
Amongst the many compounds isolated from guggulresin were two steroids: E and Z- guggulsterones.^{114, 115} This ethyl acetate soluble fraction was named guggulipid. A joint patent involving Sukh Dev, then Director of Malti-Chem Research Centre, Baroda, and CDRI was filed. The lipid lowering action of guggulsterone was shown to be mediated through activation of lecithin cholesterol acyltransferase (LCAT) and tissue lipolytic enzymes, enhanced catabolism of LDL, increased faecal bile acid excretion, in-

Figure 1: Guglip, a commercially standardized herbal preparation



hibition of cholesterol biosynthesis in hyperlipidaemia without causing any adverse effect of plasma HDL.¹¹⁶

Guggulsterone has been reported to possess mild antioxidant activity together with lipid lowering action. The protection provided by guggulsterones against oxidative changes in human LDL is due to its free radical scavenging property, as this compound significantly inhibits the generation of hydroxyl radicals. Guggulsterones stimulated LDL receptor mediated catabolism of LDL in treated rats.



Because the order of activity of the guggulipid fraction and that of the pure E and Z- guggulsterones is almost the same, although the proportion of these guggulsterones in the extractive was only about 4.4 per cent, it was therefore, decided to develop a guggulipid fraction itself into a hypolipidaemic agent.

After considerable study, both a bio-assay and chemical assay methods were developed. The bioassay was based on testing the hypolipidaemic activity in triton-induced hyperlipidaemic and alcohol-induced hypertriglyceridaemia in rats, while chemi-

cal assays were based on TLC separation and UV spectrophotometric determination and HPLC estimation method for E and Z- guggulsterones. A large number of samples of gum guggul collected from different regions of the country and stored for varying periods were investigated to standardize the drug guggulipid.

Clinical trials

Clinical trials conducted by CDRI scientists showed that guggulipid reduced serum cholesterol and triglycerides by an average 24 per cent and 22 per cent, respectively, and that some 260 of a group of 330 hyperlipidaemic patients responded to treatment. The results clearly indicated the efficacy of guggulipid and that it was tolerated better than clofibrate, the alternative available drug. Based on these results, in 1986, the Drugs Controller of India gave permission to market guggulipid as a new drug.

Transfer of technology

An agreement for licensing the product to Cipla Ltd., Bombay, was signed in January 1987, and the technology was formally released to the company by the former Prime Minister, Mr Rajiv Gandhi. Commercial production of guggulipid began in October 1987. It was marketed in the form of tablets and granules under the trade name Guglip (Figure 1) and also exported.

Since the preparation is a standardized extract of an Ayurvedic medicine and has been developed as a modern medicine, it was designated by the licensee firm as an "allovedic" drug, that is, a combination therapy incorporating concepts of modern medicine and Ayurveda. The drug was well received; and the demand shortly after its release showed an upward trend.

Gugulipid was commercialized at 10 tonnes per annum and from 1996 to 1997 its annual sales value was estimated at about US\$ 80,000. Based on the proceeds from these sales, CDRI received a good financial return in terms of royalty payments. However, owing to the unavailability of the gum guggul raw material, demand for

the drug could not be met and its production was discontinued in July 1997. Since then, CDRI has developed a technique for manufacturing the active guggulsterones. This technology has now been licensed to a company for commercialization.

Case study: ProMind

Traditional knowledge and research

Bacopa monniera (Linn) Pennel, Syn: *Herpestis monniera* (Linn) HB&K (Vernacular: Brahmi, Aindri, Jal Neem), is a perennial creeper found throughout India in wet, damp and marshy areas. In the folklore of Indian medicine, *Bacopa monnieri* is used as brain or nerve tonics.

This plant has been mentioned in India since the time of *Athar-Veda* (c.800 BC). The first, clear reference to its effect on the intellect and the memory is to be found in *Caraka Samhita*, written in the first century AD. It is commonly given to infants to boost memory power intelligence and mental health. *Bacopa monniera* ethanolic extract exhibited facilitatory effects on the mental retention capacity in experimental models.^{117,118}

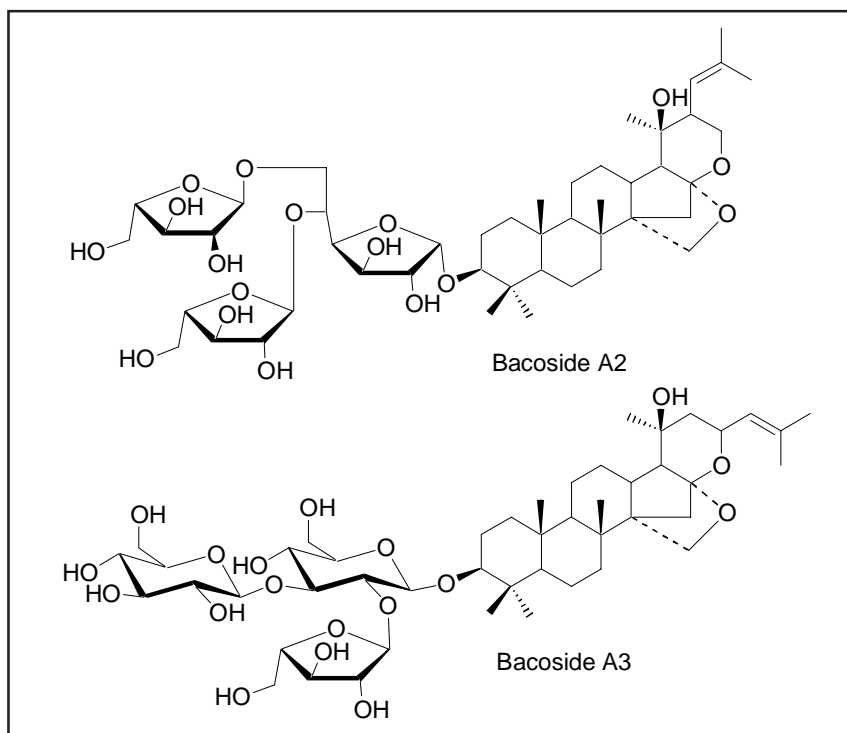
Chemistry

Evaluation of the traditional claims of *Brahmi* was initiated by investigating the effect of the ethanolic extract on the acquisition, consolidation and retention of three newly acquired behavioral responses in albino rats.¹¹⁹ The activity was localized in a fraction containing dammarane type triterpenoid saponins, designated as bacosides A and B. Both bacosides A and B showed a single spot on TLC over silica gel. Later investigations revealed that bacoside A was a mixture of three compounds, which were designated A2, A2' and A3. The sugar moiety consisted of glucose and arabinose. Repeated chromatography of bacoside A over normal and reverse phases yielded two saponins, A2 and A3, in pure form. The structures of bacosides A2 and A3 were established.^{120,121} Bacoside B was found to be a triglycoside having the same aglycone as A2 and two units

of glucose and an arabinose as sugar moiety.

Seasonal variation of bacosides

Methods have been developed for a quantitative determination of bacoside A content in the extract by UV spectrophotometry¹²² and HPLC.¹²³ To monitor seasonal variations of bacosides, fresh plant material was collected every month, extracted with ethanol and fractionated. This was carried out over a period of 14 months commencing March 1993. The TLC of the n-butanol fraction of the ethanolic extractives of the plant was used to monitor bacosides. From this study it was concluded that bacoside A (mixture of bacosides A2, A2' and A3) predominates in March and April whereas both bacosides A and B are available in May. In the remaining months, other compounds appear and disappear.



Pharmacological evaluation

Both the crude extract and the bacosides were evaluated for their nootropic activity. In adult male rats, the extract (40 mg/kg.p.s) was given 3 hours before the test in acute studies and every third day in chronic experiments. A labile test utilized brightness discrimination reaction in a semi-automatic Y-maze. In this test significant effects were observed on all the three tempero-spatial parameters - acquisition, consolidation and retention.

The initial test for stable behaviour was an active conditioned flight reaction using a sound cue. In this test also the animal learnt to escape foot shock quickly (6 days *versus* 10 days in control) and the reaction time was significantly lower from day 4 onwards.

The final test was the continuous avoidance test. A stable baseline behaviour was achieved by day 20 in this test but did not happen in the control animals.¹¹⁹ Detailed studies were carried out with bacosides A and a mixture of bacosides A and B.¹²⁴

The labile test was the brightness discrimination reaction for the Y-maze test as was done with the extract. The stable test included the active conditioned avoidance test used earlier and

a conditioned aversion test employing aversion to lithium chloride in a water-deprived rat as the cue. The bacosides produced a dose-related effect similar to that of the extracts in all the three tests. The effect of 10 mg/kg bacosides was equal to that of 40 mg/kg of extract. A dose of 10 mg/kg of bacosides given orally 60 minutes prior to testing was able to abolish both the deficits of the W-shaped curve.¹²⁵

These results suggested that the facilitatory effect of bacosides is mainly due to their ability to consolidate the retention of learnt behaviour of the earliest form, i.e. short term memory. This facilitatory effect persisted when the other two longer lasting forms of memory were getting consolidated. The anti-amnesic activity of bacoside was also evaluated. A significantly higher dose (20 mg/kg.p.c. for 3 days) was needed to prevent retrograde amnesia caused by electro-convulsive seizure, immobilization or scopolamine in the Y-maze test.¹²⁶ The bacosides on the other hand exhibited significant anti-stress activity.

Safety evaluation

The LD₅₀ of the ethanolic extract has been determined in rats and mice by

the oral and IP routes. It was greater than 3g/kg by the oral route in the species. The LD₅₀ by the IP route was 205 mg/kg (range 230-182) in rats and 224 mg/kg (range 260-135) in mice. The oral LD₅₀ of bacosides in mice was 774 mg/kg. It was evident that the extract had less acute toxicity bacosides.

Further studies have therefore been performed with the standardized preparation. Chronic toxicity studies have been performed following oral administration of 2.5, 5 and 10 times the effective nootropic dose of the preparation in a rodent (rat) and a non-rodent species for 90 days. Various hematological, biochemical and gross behavioral changes were regularly recorded. Terminal autopsy was followed by gross and microscopic examination of all viscera as per regulatory requirements. The preparation was found to be safe and devoid of any teratological effects in 2 species (rat and rabbit) and mutagenicity in *in vitro* and *in vivo* tests.

Pre-clinical neuropharmacological studies demonstrated that both *Brahmi* extract and bacosides improved short term and intermediate memory, thus improving long-term memory. Based on these wide-rang-

Figure 2: ProMind - a commercially available standardized herbal preparation



ing studies, the Drugs Controller of India granted CDRI permission to conduct Phase I clinical trials in healthy human volunteers.

The Institute has assessed the safety and tolerability of the standard preparation in 51 healthy human volunteers.¹²⁷ Single oral doses of 20-200 mg or 100 and 200 mg once daily for four weeks were found to be safe, and did not produce any reaction or side effects. Subsequently a placebo-controlled double-blind phase II clinical trial in 36 children of Attention Deficit Hyperactivity Disorder (ADHD) was conducted. The children received either a placebo or 50 mg of the preparation twice daily for 12 weeks. The children receiving the preparation showed significant improvement in scores in several test systems and there were no side effects.

Transfer of technology

As the trials did not reveal any undesirable effects, a decision was taken to market a standardized fraction of the plant extract as a herbal drug. The preparation containing a standardized extract of *B. monniera* was licensed to a private company, which commercialized the product under the trade name Memory Plus. The product was formally launched in February 1996 by the then Prime Minister, Mr. Narsimha Rao, and was successfully marketed and exported to several countries. In 2001, the product was licensed to another company, Lumen Marketing, Chennai, India, which commercialized the product in 2002 under the name ProMind (Figure 2).

Conclusion

CDRI provides a leading role in developing India's science and technology capability in drug research and development; undertaking frontline research; and providing specialized science and technology services and human resource development. Indeed, the CDRI drug development programme relies heavily on traditional knowledge of herbal remedies- a practice now vigorously followed by international pharmaceutical companies. The two case studies, selected from a set of several cases of a CDRI research project on technology transfer, present a successful implementation of technology transfer initiative.

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