

Abundance from the hive

Apis Flora invests in R&D
to obtain high-quality products
made from propolis

Yuri Vasconcelos

Anyone who has ever tried to treat a cold or sore throat with medicines made with honey or propolis, the resin produced by bees to seal and sterilize hives, has come across products made by Apis Flora. The company, headquartered in Ribeirão Preto, in inland São Paulo State, has been a Brazilian pioneer in the manufacture of products containing these two substances. Established in 1982 by agronomist Manoel Eduardo Tavares Ferreira and chemist Antônio Carlos Meda, Apis Flora has a portfolio of over 100 products including medicines, food items, pharmaceutical supplies and personal hygiene products. It is present in all Brazilian states and has more than 6,000 points of sale, mainly in pharmacies and natural products shops. Of the company's 85 employees, 15 are in the department of research. "Our team is made up of chemists, pharmacologists, biologists, biotechnologists and biomedical professionals. The department of research has six individuals with PhDs, one with a master's degree, one pursuing her master's and two with undergraduate degrees," says Andresa Aparecida Berretta e Silva, manager of research, development and innovation (RD&I) at Apis Flora.

Experiment to obtain nanostructured systems containing propolis



COMPANY

APIS FLORA

R&D Center
Ribeirão Preto, SP

Number of employees
85

Principal products
Medicines and
foods made from
honey and propolis



Researchers at Apis Flora:
Franciane Oliveira, Juliana
Hori, Hernane Barud,
Andresa Berretta and
Andresa Rodrigues

The company's strategy for creating more sophisticated products is embodied in its investment in research and establishment of university partnerships to develop projects in api-phytotherapeutics. "From the time the company was established to the mid-1990s, we were focused on less complex products such as syrups and compounds of honey, royal jelly and mouth sprays made with propolis. We invested in R&D and are currently studying and developing more-technologically-advanced products, such as propolis microparticles," says Raul Ferreira, manager of planning and new business. These microparticles, produced through the process of spray drying, then undergo microencapsulation, which maintains the stability of the active components.

Created in the Apis Flora laboratories, the propolis microparticles have become

top-selling exports. Each year, nearly 18 metric tons of the product are sold to a customer in China, whose name has to remain confidential for business reasons. "Last year, we filed a patent related to the microparticle manufacturing process," Ferreira says.

Apis Flora sells its products to customers in 15 other countries in addition to China, including the United States, Canada, Japan, South Korea, and Argentina. Last year exports accounted for 15% of its R\$25 million in sales revenue. In Brazil, the company provides industrial inputs to large companies in the pharmaceutical, cosmetics and food industries, such as Johnson & Johnson, Unilever and L'Oréal. "We're a certified supplier of Johnson & Johnson for their infant line made with honey. For L'Oréal, we sell propolis extract and lyophilized

[freeze-dried] royal jelly," Ferreira adds.

The company is no stranger to the development of innovative products such as microparticles. Back in the 1980s, Apis Flora began to explore the potential of propolis extract, a substance known for its anti-inflammatory, wound-healing and antimicrobial properties. It also created a new line that combined honey, propolis extract and medicinal plants into a single product. "In 1983, when we started manufacturing our propolis extract, Brazil had no regulations governing propolis. We worked with the Ministry of Agriculture to draft the Technical Regulation of Propolis Identity and Quality (TRPIQ), published in 2001,



At the company laboratory, tests on medications and growing bacteria for experiments

that deals with its standardization,” says Raul Ferreira.

Apis Flora’s first university partnership came about as a result of its need to learn about the chemical composition of Brazilian propolis, and thus establish quality parameters. “About 20 years ago, we approached Professor Jairo Kenupp Bastos of the School of Pharmaceutical Sciences at the University of São Paulo (USP) in Ribeirão Preto, to get his help on this characterization. Up to that time, there were no studies of this kind in Brazil,” says Andresa Berretta, who has worked at Apis Flora for the past 16 years and was responsible for the company’s first more complex product:

Propolis is undergoing tests on medicines to fight arthritis, candidiasis, and burns and in ethanol production

a medicine produced from propolis extract that treats skin wounds and burns.

The product formulation occurred as Berretta was pursuing her master’s and doctorate at USP in Ribeirão Preto. She explains that it is a thermoreversible cold fluid that becomes a gel when it comes in contact with the skin – whose

temperature is higher – relieving pain and forming a protective layer against outside agents. “We conducted tests on animal wounds as well as clinical trials on 32 patients at the Hospital das Clínicas in Ribeirão Preto, led by Professor Werther Marchesan. The medication is awaiting registration by ANVISA (Brazilian Health Surveillance System) before sales can begin,” she says. This work gave rise to two of the six patent applications the company has filed with the Brazilian Industrial Property Institute (INPI).

BIOCELLULOSE BANDAGES

Another company innovation is a bio-bandage made using biocellulose membranes impregnated with propolis extract – biocellulose is the name given to cellulose produced by bacteria. This study is being conducted together with researchers from the Chemistry Institute of the São Paulo State University (Unesp) in Araraquara, in partnership with Professors Younés Messaddeq (currently at Laval University, Canada) and Sidney José Lima Ribeiro. “Our biobandages treat hard-to-heal wounds such as burns and chronic skin ulcers. They shorten

NAMES OF INSTITUTIONS FROM WHICH APIS FLORA RESEARCHERS GRADUATED

Andresa Aparecida Berretta e Silva, farmacêutica, pharmacologist, Manager of Research, Development and Innovation	University of São Paulo (USP/Ribeirão Preto): Undergraduate, Master’s, Doctorate and Post-doctoral research
Andresa Piacuzzi Nascimento Rodrigues, biologist, coordinator of the microbiology laboratory	University of Franca (Unifran): undergraduate degree; University of São Paulo (USP/Ribeirão Preto): Master’s and Doctorate
Franciane Marquede de Oliveira, pharmacologist and researcher	Fundação Hermínio Ometto (FHO-Araras): Undergraduate degree; University of São Paulo (USP/Ribeirão Preto): Master’s, Doctorate and Post doctoral research
Hernane da Silva Barud, chemist and researcher	Federal University of Juiz de Fora (UFJF): Undergraduate degree; São Paulo State University (Unesp/Araraquara): Master’s and Doctorate Trent University (Canada): Postdoctoral research
Juliana Issa Hori, biologist and researcher	Federal University of São Carlos (UFSCar): Undergraduate degree; University of São Paulo (USP/Ribeirão Preto): Master’s Doctorate and Post-doctorate



Cellulose biomembranes obtained from bacteria of the genus *Komagataeibacter*, used to treat wounds. Above, a powder with microparticles masks the flavor and ensures stability of the propolis

treatment time and the pain of patients who suffer from first- and second-degree burns,” says chemist Hernane Barud, project coordinator. According to Barud, who is also a professor at the University Center of Araraquara (Uniara), animal tests and human clinical trials have been conducted under the leadership of Professor Marco Andrey Cipriani Frade of the School of Medicine at USP in Ribeirão Preto. The team is currently in the process of completing ANVISA registration. This is one of the Apis Flora projects that has received funding from the FAPESP program for Innovative Research in Small Businesses (PIPE).

Propolis extract can also be used in developing a mucoadhesive gel to treat the fungal infection vaginal candidiasis. “This project also has the involvement of Professor Gustavo Goldman of the School of Pharmaceutical Sciences of Ribeirão Preto. It was he who conducted the study that explained propolis’ antifungal mechanism of action,” Berretta explains. In March 2016, clinical trials of the new product will begin with patients at the HC [Hospital das Clínicas] in Ribeirão Preto,” she says.

The company is also using propolis to develop a medication for rheumatoid arthritis, a disease that can lead to deformity and destroy joints. “Our group was the first to show how propolis acts to inhibit one of the principle channels of inflammation of cells known as inflammasomes, which are related to autoimmune diseases, including rheumatoid arthritis,” says Juliana Issa Hori, biologist and coordinator of the company study. This project is being carried out in collaboration with Professor Thiago Mattar Cunha at the USP School of Medicine in Ribeirão Preto.

In another line of research, propolis extract is being studied for its use as a possible solution to problems encountered by producers of sugarcane and alcohol. “Bacterial contamination of alcohol fermentation processes is common and causes huge economic losses. To prevent this, distilleries use antibiotics,” explains Andresa Piacuzzi Rodrigues, who heads up Apis Flora’s microbiology laboratory. The problem with using antibiotics is that they leave residue in the sugar and alcohol as well as in other byproducts of the fermentation.

Apis Flora is also developing phytotherapeutic products from Brazilian biodiversity. The project led by pharmacologist Franciane Marquede de Oliveira, a company researcher, is attempting to create a medicine based on the *louro-de-cheiro* (*Ocotea duckei*) to treat tegumentary and visceral leishmaniasis, diseases endemic to Brazil caused by protozoa of the genus *Leishmania*. “Standard drugs present a number of side effects that cause many patients to abandon treatment,” Oliveira says. “We have developed a nanotechnology-based system that releases the components of *Ocotea duckei* into the infected tissue,” she says. “We detected the components with anti-leishmaniacal effect and established a system to carry them throughout the body and we are currently conducting initial *in vivo* tests and human trials.” ■

Projects

1. *Development of a nanotechnology based medication from Brazilian biodiversity to treat leishmaniasis* (No. 2014/50410-3); **Grant Mechanism:** Innovative Research in Small Businesses (PIPE); **Principal Investigator:** Franciane Marquede de Oliveira (Apis Flora); **Investment:** R\$102,547.75.
2. *Development of a medication to treat vulvovaginal candidiasis* (No. 2013/50496-2); **Grant Mechanism:** Innovative Research in Small Businesses (PIPE); **Principal Investigator:** Andresa Aparecida Berretta e Silva (Apis Flora); **Investment:** R\$425,262.37 and US\$123,911.50.
3. *Obtaining a natural antibiotic through biotechnological process to control contamination of alcohol fermentation processes* (No. 2012/50215-0); **Grant Mechanism:** Innovative Research in Small Businesses (PIPE); **Principal Investigator:** Andresa Piacuzzi Nascimento Rodrigues (Apis Flora); **Investment:** R\$124,724.00 and US\$6,500.00.
4. *Development and analysis of biobandages obtained from bacterial cellulose and standardized propolis extract (EPP-AF) for the treatment of burns and/or skin lesions* (No. 2011/51725-0); **Grant Mechanism:** Innovative Research in Small Businesses (PIPE); **Principal Investigator:** Hernane da Silva Barud (Apis Flora); **Investment:** R\$273,525.00.