



**UNIDO Session on Utilization of plants for non-food uses:
Challenges and perspectives – March 3, 2010**

Biodiversity-related Bioenterprise Development in Brazil

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Contributed by  **EXTRACTA**
MOLÉCULAS NATURAIS S/A



BIODIVERSITY-RICH REGIONS IN BRAZIL



- **22% of the Global Plant Biodiversity**
- **>60.000 Species of Vascular Plants**

Under 1500 plant species are documented by Brazilian Traditional Medicine (W.B.Mors et al, 2000). Could modern bioresearch and biotechnologies add significantly to potential pharmaceutical value?



ADDING PHARMA VALUE TO BIODIVERSITY

■ **REGULATORY BACKGROUND**

- CBD and National Control over Biodiversity
- Brazilian Legislation: CGEN/MMA and Special License for Collection
- Landholder Informed Consent prior to Access

■ **ASSEMBLING THE SCREENING COLLECTION**

- Expeditions, Extraction Centers, Dry and Fluid Extract Storage

■ **SCREENING**

- Target Selection, Bioassay Development, High Throughput Screening

■ **DEVELOPMENT OF PROVEN BIOACTIVES**

- Phytotherapeutics and Pure Molecules: funding of Innovation in Brazil

■ **HARNESSING RAW MATERIAL SUPPLY & RETURN OF BENEFITS**

- Plant Identification, selection, multiplication and agro-forestry

■ **TOWARDS THE MARKET**

- Medical Ethics and Regulatory Control by National Agencies
- Pre-Clinical and Clinical Testing
- Licensing and Commercialization Contracts: IP Issues



ASSEMBLING
THE SCREENING
COLLECTION

THE EXTRACTA BANK OF CHEMICAL BIODIVERSITY

- 215 Expeditions covered ~10,000 km² of Atlantic and Amazonian Rainforests in Brazil during 1999-2010.
- 4,905 Plants were collected under rigorous control (GPS, local Digital Photos, Herbarium Samples). Small extraction samples from plant parts averaged 2.5kg.
- 11,586 TLC-controlled etanolic extracts yielded 31,492 chemical samples for HTS screening campaigns against targets of interest.
- A unified data base controls the Bank and screening results (proprietary software).



ASSEMBLING THE SCREENING COLLECTION

**RIO DE JANEIRO & BELÉM:
extraction facilities can process up to 15.000 extracts per year**

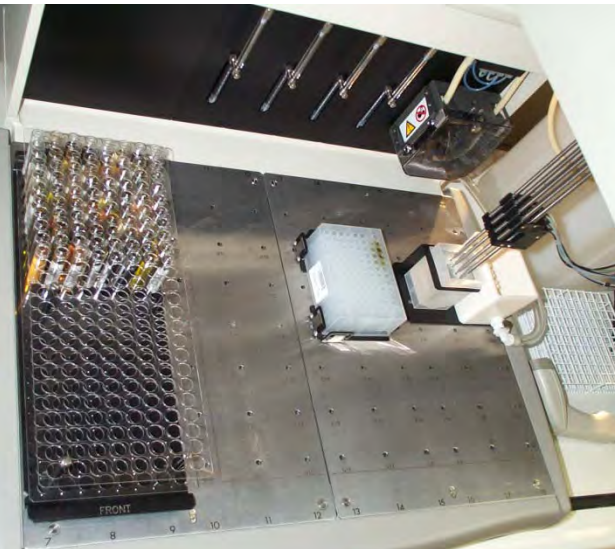


**Extracts
are stored
at – 30°C
until use**



TARGETTING BIOACTIVITY WITH HTS SCREENING

- **Target choice and Bioassay development**
 - **Extract dispensation in 96-well plates**
 - **Plate reorganization after HTS results (“cherry picking”)**





TARGETTING BIOACTIVITY WITH HTS SCREENING

Medium-High Throughput Screening at EXTRACTA: up to 24,000 operations daily





TARGETTING BIOACTIVITY WITH HTS SCREENING

Target: xxxx
 Assay: xxxxx
 Run: Screening 1
 RI: xxxxx
 DIPlateID: 100055
 2 assay plates in duplicate
 Z' factor: 0.951
 X²: 8.1
 RI Ic50: 2.7e-5 M

DATA PROCESSING

Banco de Biodiversidade - Mozilla {Build ID: 2001092408}

100055_580_0 Download: PNG Graph 8 de 36 Anterior | Próxima

Sumário de Processamento

Datasets	2
CP:1	100 (1.61)
CN:1	-2.17e-19 (0.0159)
S	78.9 (18.6)
Z'-factor	0.291
Z''-factor	0.951
RC:1 Chi Sq	8.11
RC:1 Ic50	2.74e-05 (1.02e-06)
RC:1 H	6.9 (1.21)
RC:1 Ymin	-0.104 (0.124)
RC:1 Ymax	89 (0.548)

Graph (ID: 100055_580_0)

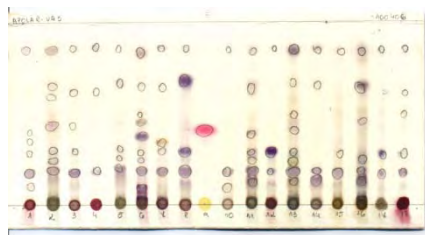
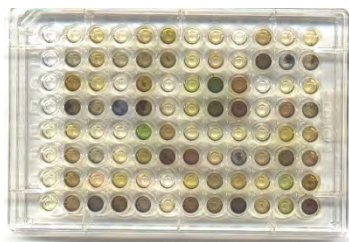
Data (ID: 100055_580_0)

	1	2	3	4	5	6	7	8	9	10	11	12
A	101 (0.325)	87.5 (2.46)	85.4 (2.03)	82.1 (1.23)	77.4 (4.98)	86 (1.49)	82.4 (0.000438)	85.9 (0.571)	88 (0.219)	83.6 (0.903)	77.9 (7.85)	0.0126 (0.0658)
B	98.6 (0.537)	90.8 (0.821)	84.2 (0.732)	85.3 (3.27)	70 (1.13)	73.9 (5.96)	30 (0.00203)	69.5 (0.675)	35.8 (2.94)	85.4 (3.12)	88.9 (0.723)	-0.0229 (0.0628)
C	102 (1.4)	82.4 (7.77)	83.6 (1.25)	87.3 (1.09)	78.5 (1.03)	86.6 (1.5)	91.5 (1.03)	79 (1.04)	92.7 (3.41)	85.5 (3.28)	92.5 (0.0573)	33.7 (3.38)
D	98.6 (0.537)	84.6 (0.892)	82.6 (6.37)	90.4 (2.46)	86.2 (2.01)	88.7 (0.344)	94.3 (2.67)	87.3 (0.345)	85.9 (7.69)	89.6 (2.06)	88.8 (0.534)	70 (5.84)
E	-0.00265 (0.00984)	85.3 (2.93)	83.1 (73.3)	85.2 (5.67)	91 (1.31)	89.3 (5.8)	92.8 (1.24)	90.8 (0.34)	86.1 (5.5)	79 (12.6)	65.8 (7.99)	84.6 (2.37)
F	0.0156 (0.00844)	80.1 (0.85)	85.4 (0.531)	88.3 (2.79)	72.4 (3.39)	95.3 (1.29)	79.4 (4.97)	67.6 (3.91)	1.4 (0.249)	85.8 (2.18)	91.6 (4.92)	88.9 (0.416)
G	-0.0103 (0.0281)	75.8 (6.99)	77.1 (8.93)	88 (1.28)	63.1 (4.87)	94.1 (6.26)	83.2 (4.82)	86.6 (5.58)	86.7 (2.8)	71.3 (7.32)	70.2 (10.1)	89.6 (0.995)
H	-0.00265 (0.00984)	77.5 (1.53)	68.3 (7.2)	2.13 (0.330)	86 (4.25)	86.8 (1.51)	5.36 (1.25)	81.7 (1.75)	82.3 (1.94)	82.1 (3.7)	81.9 (5.1)	90 (1.88)

Document: Done (18.536 secs)



BIODIRECTED CHEMICAL FRACTIONATION



- 1) Tannin score and removal
- 2) TLC quality control
- 3) Biodirected Extract fractionation in search of active compound

- UV-visible spectrometry and HPLC

- **High definition LC-MS**

- **NMR experiments**

- **X-Ray Crystallography**



OUTSOURCED





EXTRACTA INNOVATION PIPELINE

EXTRACTA PIPELINE: POSITIVE HITS NEED FINANCING TO RETURN BENEFITS LOCALLY

Target Pathology	Primary Bioassay	No. of Strongly Active Extracts	No. of Purified Active Fractions	Pure Isolated Compounds (95% Pure)	
Resistant Hospital & Community Infections	Meticillin - resistant S.Aureus (MRSA)	49	12	3	
Disease (DPOC)	Elastase inhibition	32	18	7	
Hepatitis C	Cell Protection against surrogate target	118	12	NA	
Chagas Disease	Cruzipain inhibition	98	2	NA	
Tuberculosis	InhA inhibition	38	13	NA	
Diabetes Type II	PTP-1B inhibition	348	NA	NA	
Development Candidate Extracts		683	<i>In yellow: Innovation Projects Financed by Brazilian Agencies FINEP and FAPERJ</i>		



ANTI-MRSA SELECTED CRUDE EXTRACTS

MIC Comparison between 3 selected extracts and a Commercial Antibiotic against Meticillin-resistant *S. aureus*

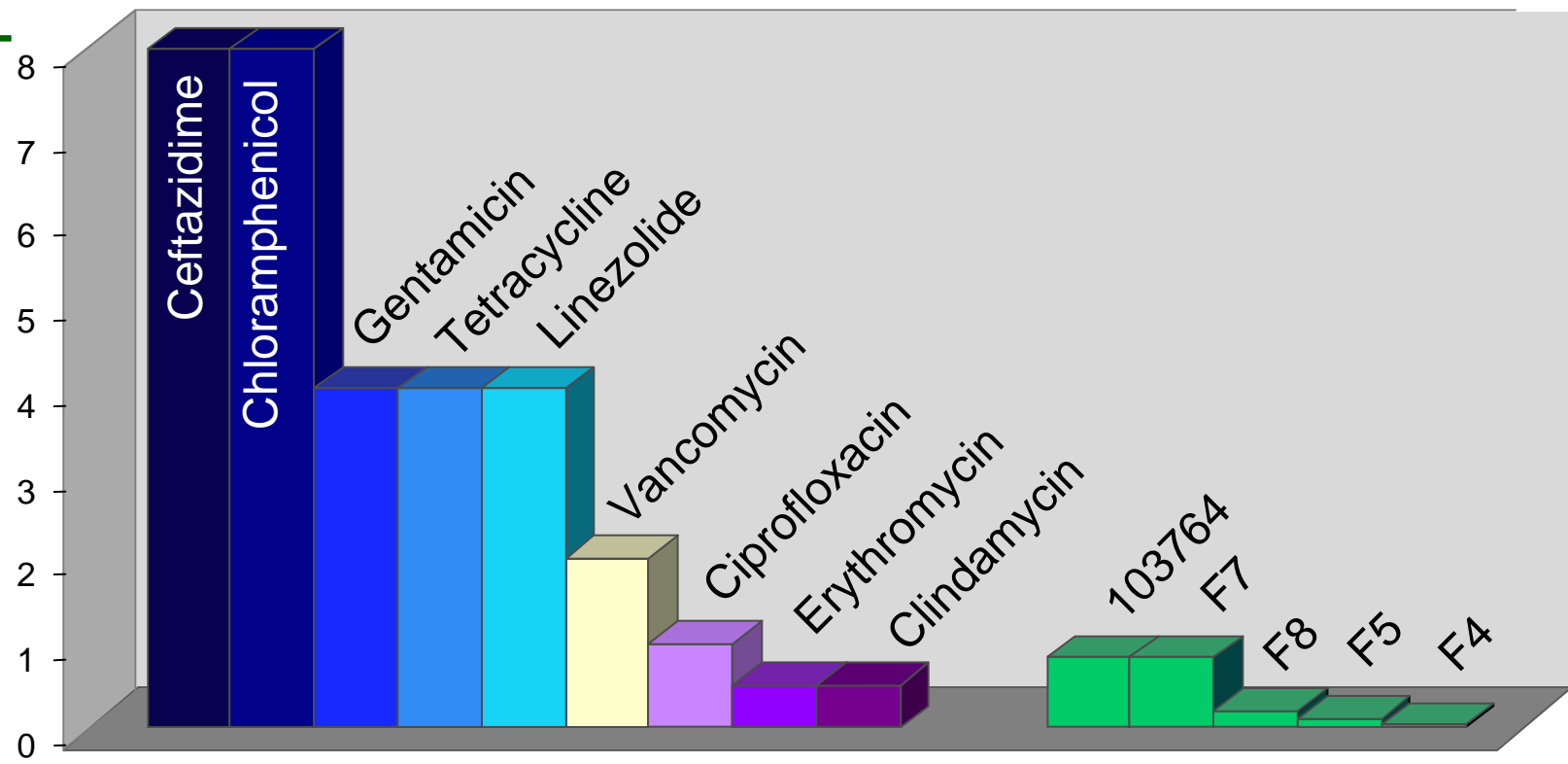




ANTI-MRSA EXTRACTS, FRACTIONS AND PURE COMPOUNDS

MIC of Extract 103764 and its purified Fractions compared to Commercial Antibiotics

MIC
 $\mu\text{g/mL}$





EASTERN AMAZONIAN
JOINT VENTURE
(ONGOING MODEL)

Public Herbarium (Goeldi Museum)

Collectors

Landholder Conserves and Grants Access to Biodiversity

Access Contracts with Promise of Future Return of Benefits

**EXTRACTA
(Licensed
by CGEN)
And UFPA**

R&D Contracts

Academia

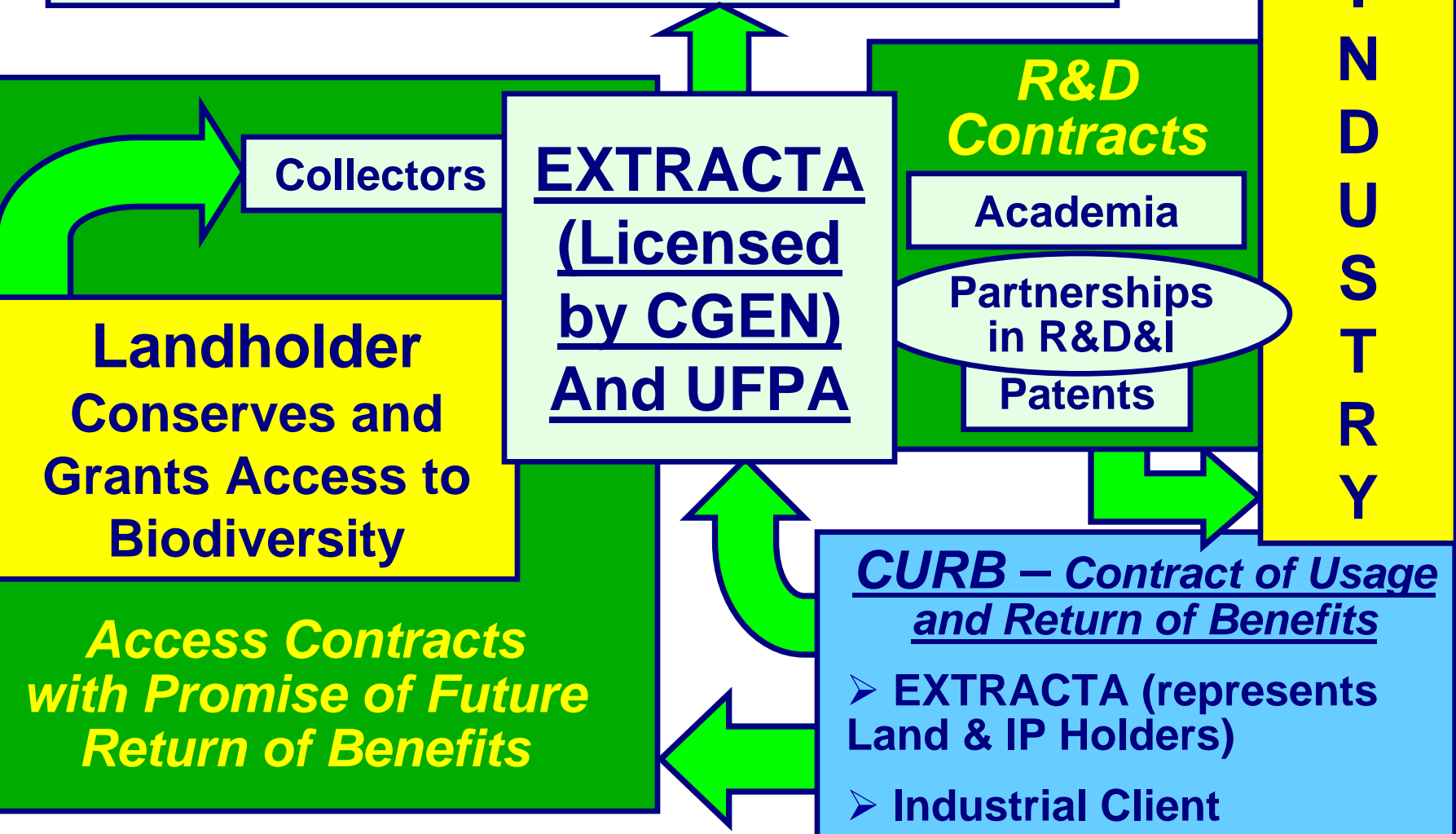
Partnerships in R&D&I

Patents

**I
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CURB – Contract of Usage and Return of Benefits

- EXTRACTA (represents Land & IP Holders)
- Industrial Client





HARNESSING RAW MATERIAL SUPPLY & RETURN OF BENEFITS (I)

■ CLONE BOTANICAL CLASSIFICATION

- Phylogeographic and biodiversity study
- Macro and microanatomical study of “plant drug”
- DNA “fingerprinting”

■ CLONE CHEMISTRY AND BIOACTIVITY

- TLC, SPE, Column Chromatography & HPLC
- Isolation of Bioactive Plant Marker (95% Purity)
- Bioactive Compound Structure

■ CLONAL MULTIPLICATION

- Agricultural (cuttings & other)
- Micropropagation by Tissue Culture



HARNESSING RAW MATERIAL SUPPLY & RETURN OF BENEFITS (II)

■ **AGRO-FORESTRY STRATEGY**

- **Agro-Forestry Engineering Plan Approval (StateLevel)**
- **Local Aclimatization of Cloned Plantlets at the Farm**
- **Farm Experimental and Producing Clonal Orchards**
- **Underbrush Enrichment with Clones in the Neighborhood of Original Plant Access**

■ **CGEN-ASSISTED INDUSTRY-EXTRACTA CURB**

- **The Important Issue of Intellectual Property in PHARMA and the Brazilian Approach to the Solution**
- **Returning Benefits to the Farmer: Engineering of Clonal Planting and Production; Commercialization Goals and Pre-Set Prices; Technology Transfer through Training of Extensionists and Farmhands**
- **Returning Benefits to Associated Institutions and Inventors**



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