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APN ASIA-PACIFIC NETWORK FOR GLOBAL CHANGE RESEARCH

Pioneering plant metabolomic library of Indonesian plants for research, conservation, capacity building and economic development

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ABSTRACT

Indonesia, one of the world's most biodiverse countries, is undergoing mass deforestation, exacerbating climate change and leading to accelerated loss of species. This project addressed the urgent need to conserve endangered Indonesian biodiversity, specifically the potentially life-saving bioactive compounds harboured within its plants. A group of Indonesian researchers from Universitas Nasional (UNAS) in Jakarta received training in RApid Metabolome Extraction and Storage (RAMES) technology, an ethical, low-impact, field-deployable and cost-effective methodology developed by Rutgers University. The team of Indonesian scientists used this technology to create the first metabolomic library of Indonesian plant species and an easily transportable collection containing 501 metabolome samples from 296 species. This pioneering and readily shareable resource aims to foster collaborative research into plant metabolomics and natural products, reaching across Indonesia and the broader Southeast Asia region. The project also facilitated four formal discussion forums, two of which were international conferences, promoting exchange among Indonesian, Southeast Asian and USA scientists, with notable participation from the Indonesian National Research and Innovation Agency (BRIN). These efforts culminated in the formation of a strategic partnership among UNAS, BRIN and Rutgers.

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HIGHLIGHTS

- Trained 34 young Southeast Asian scientists in using RAMES-STN technologies.
- Created the first metabolomic library of Indonesian plants containing 296 species.
- Held two practical training workshops and two international conferences.
- Signed MOU for collaboration between UNAS, BRIN and Rutgers University.

1. INTRODUCTION

Plants are critical for human survival. Their contributions to humanity stretch from producing oxygen to forming the foundation of all food chains – including those required for human nutrition. Plants also provide an indispensable foundation for all ecosystems on Earth. Many plant-derived natural products have been converted into human medicines – with up to 70% of all drugs currently in the market being inspired by nature (Newman & Cragg, 2016) – as well as crop protection agents, dietary supplements, cosmetic ingredients, preservatives, disinfectants, flavours, fragrances, colourants and sources of fibre (Schmidt et al., 2007). This breadth of contributions arises from plants being exceptional sources of functional molecular diversity and novel molecular chemotypes, fine-tuned over millions of years of evolution to offer protection against stresses, diseases, and predators (Dixon, 2001). It has been demonstrated that existing plant species harbour a much greater diversity of bioactive metabolites than any synthetic chemical library ever created (Schmidt et al., 2007). Conservation of plant biodiversity is, therefore, crucial for the wellbeing of humankind and fundamental to the health of all ecosystems on Earth.

However, this critical link between biodiversity and human health is under grave threat due to the ongoing sixth mass extinction event driven by anthropogenic global climate change (Ceballos et al., 2015). Current estimates indicate that around one million plant and animal species are at risk of extinction within decades, marking a crisis unparalleled in human history with potentially catastrophic global ramifications (IPBES, 2019). Concerning flora alone, it is estimated that out of the approximately 450,000 species of flowering plants in existence, a third is at risk of extinction (Pimm & Joppa, 2015). Habitat destruction and deforestation are the major causes of plant extinction, particularly pronounced in the world's most biodiversity-rich, tropical regions (Estoque et al., 2019). In addition, projections suggest that without radical reductions in carbon emissions, "there will be further acceleration in the global rate of species extinction, which is already at least tens to hundreds of times higher than it has averaged over the past 10 million years" (IPBES, 2019). The urgency of the situation cannot be overstated and there is an immediate need for interdisciplinary adaptation measures, along with stringent policy changes promoting mitigation efforts worldwide.

Indonesia, one of the world's most biodiverse countries (CBD, n.d.), is a prime example of a country rapidly losing its biodiversity. This archipelago includes two global biodiversity hotspots, Sundaland and Wallacea (CBD, n.d.). It houses the largest coral reef area in Southeast Asia (ADB, 2014), most of the world's tropical peat forests (Posa et al., 2011), the world's largest mangrove forest area (Basyuni et al., 2022), and about 15.5% of the entire world's flora, including approximately 80,000 species of spore plants and 30–40,000 seed plants (Maskun et al., 2021). The country also exhibits remarkable endemism, with roughly 40–50% of flora species specific to each island, except for Sumatra (Maskun et al., 2021). However, Indonesia's rapid population and economic growth, coupled with deforestation caused by fires, rampant logging, and the expanding palm oil industry, are causing swift habitat degradation, which, alongside climate change, pollution and alien species, severely threaten this crucial biodiversity (CBD, n.d.; Cleary & DeVantier, 2011).

Therefore, there is an urgent need to protect and catalogue Indonesia's biodiversity and the biochemical compounds contained by its endangered flora since the irreversible loss of potentially life-saving drugs and other bioactive compounds derived from these species would represent an immense tragedy for human health and wellbeing. To address this challenge, particularly given that many of these ecosystems are in countries in need of scientific capacity development, Rutgers scientists developed RApid Metabolome Extraction and Storage (RAMES) technology (Skubel et al., 2018). This is an ethical, innovative, simple, rapid, highly cost- and space-efficient method to efficiently catalogue and preserve the metabolome and genome of living organisms using compact glass fibre discs as a physical platform. It is also fully field-deployable and highly sustainable, as it requires sampling just two grams of fresh tissue, minimising plant damage. This technology is complemented by a liquid chromatography mass spectrometry (LC-MS)-based method to generate a complete metabolomic signature for each plant sample, supporting chemodiversity studies and taxonomic identification. Functional analysis of RAMES samples can be performed using a fully compatible set of Screens-To-Nature (STN) bioassays designed for simple, compact, low-cost and portable assessment of bioactivity of RAMES samples. These pioneering technologies have been validated and described in full detail by Skubel et al. (2018).

In Stage 1 of this project, selected Indonesian researchers received theoretical and practical training in natural product research methodologies, including RAMES and STN technologies. In Stage 2, the researchers applied this knowledge to create the Indonesia Metabolome And Genome Innovation and Conservation (MAGIC) Library, the first metabolomic collection of Indonesian plant species. The project's commitment to promoting health, education, innovation, and biodiversity conservation is aligned with Sustainable Development Goals 3, 4, 9 and 15.

2. METHODOLOGY

2.1. Hybrid training of Indonesian researchers

2.1.1. Online training

The training of Indonesian participants was initiated in the context of the Center for Botanicals and Chronic Diseases, or CBCD (https://cbcd.rutgers.edu/), funded by the USA National Institutes of Health – Fogarty International Center (NIH–FIC). This centre provides training across a spectrum of research disciplines, spanning basic biomedical and botanical to clinical and applied sciences, including translational and implementation science. Using a culturally sensitive approach, it connects traditional use of botanical therapeutics in participating countries – Indonesia and Tajikistan – with the state-of-the-art research capabilities of participating USA institutions. By combining intensive training in Western science with robust knowledge of traditional botanical medicines, the CBCD program aims to cultivate scientists who can integrate these two systems for the benefit of participating countries. Instruction is organised around four sections: Ethics Core, Botanical Core, Analytical Core and Drug Development Core.

The 2021–22 cohort of the program included twenty-five trainees, sixteen of whom were Indonesian from three Indonesian institutions. Trainee selection considered academic performance, English proficiency, and personal research interests. Training was conducted online using the Rutgers University Canvas learning management system and Zoom video conferencing, avoiding disruption by the global COVID-19 pandemic lockdowns. A mix of synchronous and asynchronous lectures was used for instruction delivery, and regular assignments and examinations were used to monitor progress and assess understanding. In addition, trainees undertook research on a self-selected topic under the guidance of mentors from Indonesia and the USA.

2.1.2. Practical RAMES-STN workshop

After the easing of COVID-19 lockdown measures, an immersive in-person full-day training workshop on RAMES and STN technologies, funded by the Asia-Pacific Network for Global Change Research (APN), took place in July 2022 in the Mt. Halimun-Salak National Park in West Java. This protected, highly biodiverse location was selected to underscore the portable character of these technologies while reinforcing the notion that our natural environment contains a vast array of phytochemicals with invaluable bioactivities. The seventeen Indonesian trainees included six PhD students, eight MS students, and three BSc students. Thirteen of these trainees were selected from the group who had already completed CBCD training, while the remaining four were candidates for the subsequent CBCD cohort.

The large number of workshop participants, as well as their heterogeneous levels of technical

FIGURE 1. **Collection sites for Indonesia MAGIC Library**. Due to budget limitations, most samples were collected from forest parks, botanical gardens, and other green spaces within the Jakarta Metropolitan Area, as well as some collection sites around the city of Bandung, and one site in Bali (not shown in map).

expertise, potential language barriers and time limitations, all posed significant potential challenges. These were addressed prior to the event through careful organisation, and all trainees were also sent a detailed manual in English and asked to read it before the workshop. On workshop day, two Rutgers scientists provided instruction in plant collection methods, including documentation and species identification. They subsequently learned the rapid preparation of ethanolic extracts and their immobilisation onto glass microfiber discs using RAMES technology and then learned to apply STN field-adapted bioassays to assess the antibacterial, antifungal, and antioxidant bioactivities of the freshly prepared extracts. All technical details about these methodologies are published in Skubel et al. (2018). After the workshop, participating researchers from Universitas Nasional (UNAS) received a kit containing all materials for performing RAMES extraction and STN functional assays on 1,000 samples.

2.2. Creation of the Indonesia MAGIC Library

2.2.1. Collection and extraction of samples using RAMES technology

The development of the pilot Indonesia MAGIC Library was spearheaded by a team of Indonesian scientists from UNAS. This team comprised three senior researchers specialising in medicinal plants, environmental sciences, and ethnobotany, along with three young scientists who had completed the training described in Section 2.1. Rutgers scientists also assisted via regular progress meetings and participation in some collection activities. The UNAS team conducted field collection trips to 25 areas of Indonesia (Figure 1, Table 1), and local guides assisted with preliminary taxonomic identification. Species selected for the library at this pilot stage were Indonesian species with previously known medicinal properties. Whenever a species of interest was preliminary identified in the field, the team took pictures of the whole plant, as well as flowers or fruits if present, and recorded its GPS coordinates prior to collecting it using scissors and placing it inside a labelled plastic bag. The collected samples were then individually photographed using a

TABLE 1. Collection sites for Indonesia MAGIC Library.

Province	Collection site	No. samples
Special capital region of Jakarta	Jati Padang, South Jakarta	73
	Srengseng City Forest, West Jakarta	44
	South Jakarta	16
	Pasar Minggu, South Jakarta	12
	Rawa Barat, South Jakarta	6
	Pejaten, South Jakarta	5
	Pondok Pinang, South Jakarta	3
	Ragunan, South Jakarta	2
	Kemang, South Jakarta	2
	Pancoran, South Jakarta	2
	Pramuka Island, Seribu Islands	1
	Jakarta total	**166**
West Java	Cilembu, Sumedang	79
	Djuanda Forest Park, Bandung	70
	Bandung	64
	Sringanis Park, Bogor	33
	Bogor Botanical Garden	32
	Bojonggede	13
	Depok	8
	Bogor	7
	Bekasi	4
	Kranji	2
	Citayam	2
	West Java Total	**314**
Banten	Tangerang	15
	Serpong	5
	Banten Total	**20**
Bali	Tabanan	1
	Bali Total	**1**
Grand Total		**501**

portable lightbox just before their extraction. These images would later be used to confirm preliminary identification using taxonomic keys.

For each species, samples were collected from various tissues – leaves, stems, rhizomes, tubers, flowers, fruits and/or seeds – depending on availability and with minimal disruption to the plants. Samples were processed using RAMES technology while still in the field to minimise phytochemical degradation during transport. For each species, two grams of plant material were extracted in 5 ml of 95% ethanol using a specially modified Dremel® cordless rotary tool. The extract was filtered and loaded onto 10 mm borosilicate glass microfibre discs, which were dried using a portable fan (Skubel et al., 2018). The process was repeated until reaching a minimum of 40 discs per sample, each holding 90 µl of extract. Dry discs were stored in resealable

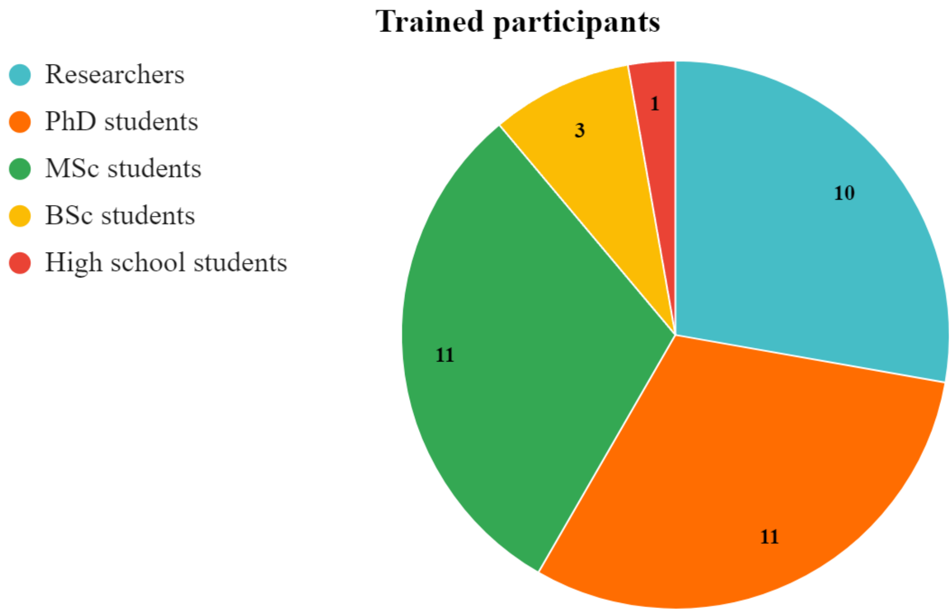

FIGURE 2. Participants trained during RAMES-STN workshops. 32 trainees were from Indonesia, 2 were from Japan, and 2 were from the USA conducting PhD studies in Indonesia. The July 2022 group was comprised by 6 PhD students, 8 MSc students, and 3 BSc students. The May 2023 group was comprised by 10 researchers, 5 PhD students, 3 MSc students, and 1 senior high-school student.

plastic bags and transported back to UNAS for storage at −20 °C.

2.2.2. *Long-term sample storage and data management*

Once dry, discs from each sample were stored in resealable plastic bags, each labelled with the corresponding unique sample ID and species name. These bags were then stored in a −20 °C lockable freezer within the UNAS laboratories.

While the library was being created, comprehensive data about each sample was kept in an Excel spreadsheet. This information included unique identification numbers, scientific and common names, family, plant organ collected, name of collector, GPS coordinates, plant habitat, whether the sample was from a cultivated species, and a summary of its traditional medicinal uses. Upon formally establishing the Indonesia MAGIC Library as a distinct unit within the Center for Medicinal Plants Research (CMPR) at UNAS, a section was created within the CMPR website to centralise all available information.

3. RESULTS AND DISCUSSION

3.1. Outcome of the hybrid training

The online training through the 2021–22 CBCD program resulted in sixteen Indonesian participants from three Indonesian institutions gaining comprehensive knowledge in phytochemistry, biostatistics, drug development, botanical supplements for human health, scientific article writing, and research ethics. After passing the theoretical component of this program, trainees acquired the required knowledge to prioritise, identify and collect plants for the Indonesia MAGIC Library. They also learnt the laboratory and data analysis skills necessary to study samples from the growing library and gained expertise for developing useful and sustainable natural-product-based medicines and consumer products. Several peer-reviewed articles have been published by CBCD trainees on their topic of research within the program (Adilah et al., 2023; Kuswandari et al., 2022; Rahayu et al., 2022; Swandiny et al., 2023).

Following the RAMES-STN practical workshop, seventeen Indonesian trainees from three Indonesian institutions, including thirteen who had already completed the CBCD training, acquired hands-on skills in plant extraction and bioactivity screening using RAMES and STN technologies (Figure 2, Table 2). The extracted flowers, leaves, and fruits revealed predominantly high antioxidant activity, with some also exhibiting low-to-moderate antimicrobial properties (results not collected as they were for demonstrative purposes).

TABLE 2. Participants trained during RAMES-STN workshops.

Workshop	Institution	Region	No. trainees
Mt. Halimun Salak National Park, July 2022	Universitas Nasional	Jakarta, Indonesia	11
	Universitas Pancasila	Jakarta, Indonesia	5
	Universitas Sriwijaya	South Sumatra, Indonesia	1
	July 2022 workshop total:		**17**
Bali Botanical Garden, May 2023	BRIN - Research Center for Plant Conservation, Botanical Gardens and Forestry	Jakarta, Indonesia	2
	BRIN - Research Center for Veterinary Science	Jakarta, Indonesia	1
	Indonesia International Institute for Life Sciences	Jakarta, Indonesia	1
	Institut Teknologi Bandung	West Java, Indonesia	1
	Jagatnatha Jembrana Botanical Garden	Bali, Indonesia	1
	Pangkep State Polytechnic of Agriculture	South Sulawesi, Indonesia	1
	Prefectural Kaiho Senior High School	Okinawa, Japan	1
	Rutgers University	New Jersey, USA	2
	Universitas 17 Agustus 1945 Jakarta	Jakarta, Indonesia	1
	Universitas Airlangga	East Java, Indonesia	1
	Universitas Nasional	Jakarta, Indonesia	2
	Universitas Sriwijaya	South Sumatra, Indonesia	3
	Universitas Udayana	Bali, Indonesia	1
	University of the Ryukyus	Okinawa, Japan	1
	May 2023 Workshop total		**19**
Total number of participants trained			**36**

While the success of the practical training was not formally assessed, the training ran smoothly thanks to detailed logistics planning and anticipation of potential problems prior to the event. Participants showed remarkable engagement and enthusiasm, even after extended periods of outdoor work (Figure 3). This was reflected in a high level of commitment, engagement, enjoyment, and productivity. These observations align with previous RAMES-STN workshops (Kellogg et al., 2010, 2016), which aim to create an engaging, exciting environment that captures the thrill of scientific discovery. Factors contributing to this include unconventional research settings, the rapid and visual nature of STN assays – such as the antioxidant test, which offers striking colourimetric outcomes within seconds – a high percentage of positive test results, and dynamic, highly experienced trainers. During the Q&A session and informal discussions which took place the day after the event, all participants voiced their positive outlook on the workshop and their intention to incorporate the learned techniques into their future research.

3.2. Significance of the Indonesia MAGIC Library

By June 2023, the UNAS team had gathered a total of 501 plant samples, comprising 296 species across 90 families (Table 3; Figure 4). The detailed list of collected samples containing all publicly available information is available on the UNAS CMPR website. Botanists at the Herbarium Bogoriense, the

FIGURE 3. RAMES-STN training workshop in Mt. Halimun Salak in July 2022.

largest herbarium in Southeast Asia, verified the identity of all species.

To our knowledge, this is the first metabolome collection of Indonesian plant species, as well as the first metabolomic library worldwide, which uses a miniaturised, physical platform. One significant advantage of RAMES technology over traditional extract libraries is storage efficiency, minimal curation time required, and potential higher compound stability during storage. Instead of being kept in liquid form, extracts are stored on lightweight, 10 mm diameter glass microfiber discs, each containing 90 µl of volume. This compact format allows the approximately 20,000 discs comprising the Indonesia MAGIC Library to fit easily in a box within a standard freezer drawer, facilitating future expansion with regard to storage and organisation. This novel format remains compatible with the application of high-throughput screening approaches for the identification of chemical leads, as the extracts can be readily eluted from the discs, enabling a range of studies from metabolite quantification to traditional bioassays.

Another key advantage of this miniaturised library is its potential for scientific collaboration and sample sharing. Unlike the glass vials traditionally used to hold extracts (Beutler, 2019), or more modern approaches using microplates (Potterat & Hamburger, 2014), these small and lightweight discs can be easily transported and shipped across large distances to researchers interested in a particular species, reducing the need for constant field collection, and saving time and natural resources. To facilitate such collaborations,

TABLE 3. List of collected plant species. As of June 2023, the Indonesia MAGIC Library contained RAMES discs loaded with ethanolic extracts from 296 different plant species.

Abelmoschus manihot	*Bougainvillea glabra*	*Curcuma amada*
Abrus precatorius	*Bougainvillea spectabilis*	*Curcuma longa*
Acalypha hispida	*Brucea javanica*	*Curcuma xanthorrhiza*
Acalypha siamensis	*Brunfelsia americana*	*Cyanthillium cinereum*
Adenanthera pavonina	*Caesalpinia pulcherrima*	*Cyclea barbarata*
Adiantum capillus-veneris	*Caesalpinia sappan*	*Cymbopogon citratus*
Aegle marmelos	*Caladium bicolor*	*Datura metel*
Aerva sanguinolenta	*Calamus rotang*	*Delonix regia*
Agathis alba	*Calliandra calothyrsus*	*Diospyros blancoi*
Ageratum conyzoides	*Calliandra tetragona*	*Dombeya × cayeuxii*
Aleurites moluccana	*Calophyllum soulattri*	*Dracaena angustifolia*
Allamanda cathartica	*Cananga odorata*	*Dyera costulata*
Allium cepa	*Canna × generalis*	*Elaeocarpus angustifolius*
Allium sativum	*Canna indica*	*Elaeocarpus ganitrus*
Allophylus cobbe	*Capsicum annuum*	*Elaeocarpus grandiflorus*
Alocasia macrorrhizos	*Capsicum frutescens*	*Elatostema calcareum*
Alpinia galanga	*Carica papaya*	*Emilia sonchifolia*
Alstonia scholaris	*Catharanthus roseus*	*Epiphyllum anguliger*
Alternanthera dentata	*Ceiba pentandra*	*Epipremnum aureum*
Alternanthera philoxeroides	*Celosia argentea*	*Eriobotrya japonica*
Alternanthera sessilis	*Centratherum punctatum*	*Eryngium foetidum*
Altingia excelsa	*Chromolaena odorata*	*Etlingera elatior*
Alyxia reinwardtii	*Chrysothemis pulchella*	*Eucalyptus deglupta*
Amaranthus spinosus	*Cinnamomum porrectum*	*Eugenia uniflora*
Anacardium occidentale	*Cissus quadrangularis*	*Euphorbia milii*
Ananas comosus	*Citrus hystrix*	*Euphorbia pulcherrima*
Angelonia sp.	*Citrus limon*	*Euphorbia tirucalli*
Annona muricata	*Clerodendrum intermedium*	*Euphorbia tithymaloides*
Annona reticulata	*Clerodendrum serratum*	*Evodia suaveolens*
Annona squamosa	*Clerodendrum splendens*	*Excoecaria cochinchinensis*
Anredera cordifolia	*Clerodendrum thomsoniae*	*Ficus ampelas*
Anthurium crystallinum	*Clitoria ternatea*	*Ficus binnendijkii*
Anthurium palmatum	*Cnidoscolus aconitifolius*	*Ficus carica*
Antidesma bunius	*Codiaeum variegatum*	*Ficus coreana*
Apium graveolens	*Coleus atropurpureus*	*Ficus elastica*
Arachis pintoi	*Coleus* sp.	*Ficus pumila*
Arenga pinnata	*Colocasia esculenta*	*Ficus septica*
Averrhoa bilimbi	*Combretum indicum*	*Finschia chloroxantha*
Averrhoa carambola	*Cordyline fruticosa*	*Fragaria × ananassa*
Bauhinia purpurea	*Cosmos caudatus*	*Gardenia augusta*
Begonia cucullata	*Costus speciosus.*	*Glochidion arborescens*
Belamcanda punctata	*Costus spicatus*	*Gmelina arborea*
Bellucia axinanthera	*Crassocephalum crepidioides*	*Gnetum gnemon*
Boesenbergia rotunda	*Crossandra pungens*	*Graptophyllum pictum*
Bouea macrophylla	*Cuminum cyminum*	*Gynura segetum*

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TABLE 3. *Continued.*

Heliconia rostrata	*Moringa oleifera*	*Pometia pinnata*
Hemigraphis alternata	*Morus alba*	*Premna oblongiifolia*
Hevea brasiliensis	*Mucuna bennettii*	*Pseuderanthemum maculatum*
Hibiscus acetosella	*Musa paradisiaca*	*Psidium guajava*
Hibiscus rosa-sinensis	*Mussaenda pubescens*	*Pterocarpus indicu*
Hibiscus sabdariffa	*Myristica fragrans*	*Pterygota horsfieldii*
Hibiscus tiliaceus	*Nephelium lappaceum*	*Pyrrosia piloselloides*
Hippobroma longiflora	*Ochna serrulata*	*Quassia amara*
Hopea celebica	*Ocimum basilicum*	*Ricinus communis*
Hylocereus costaricensis	*Ocimum tenuiflorum*	*Rivina humilis*
Hymenocallis littoralis	*Oncus esculentus*	*Rosa hybrida*
Impatiens balsamina	*Ophiorrhiza mungos*	*Rosmarinus officinalis*
Ipomoea batatas	*Orthosiphon aristatus*	*Ruellia napifera*
Ixora chinensis	*Pachystachys lutea*	*Ruellia simplex*
Juglans major	*Palaquium rostratum*	*Ruellia tuberosa*
Justicia gendarussa	*Pandanus amaryllifolius*	*Salacca zalacca*
Kaempferia galanga	*Parkia speciosa*	*Santalum album*
Kalanchoe pinnata	*Passiflora quadrangularis*	*Sauropus androgynus*
Khaya anthotheca	*Pelargonium graveolens*	*Schima wallichii*
Kigelia aethiopica	*Pemphis acidula*	*Senna siamea*
Lactuca sativa	*Peperomia pellucida*	*Sesbania grandiflora*
Lagerstroemia loudonii	*Persea americana*	*Sida rhombifolia*
Lantana camara	*Phaleria macrocarpa*	*Sinningia spesiosa*
Laportea interrupta	*Philodendron rugosum*	*Solanum betaceum*
Laportea stimulans	*Phoenix dactylifera*	*Solanum diphyllum*
Leea aequata	*Phyllanthus urinaria*	*Sonchus oleraceus*
Leucaena leucocephala	*Phymatosorus scolopendria*	*Spathodea campanulata*
Lithocarpus platycarpus	*Physalis angulata*	*Spathoglottis affinis*
Lunasia amara	*Pilea cadierei*	*Spondias dulcis*
Macaranga tanarius	*Pilea trinervia*	*Spondias pinnata*
Maesopsis eminii	*Piper betle*	*Stachytarpheta jamaicensis*
Malvaviscus penduliflorus	*Piper caninum*	*Sterculia foetida*
Mangifera indica	*Piper ornatum*	*Sterculia javanica*
Manihot esculenta	*Piper pellucida*	*Stevia rebaudiana*
Manilkara kauki	*Piper sarmentosum*	*Streblus asper*
Manilkara zapota	*Pistia stratiotes*	*Strobilanthes dyeriana*
Maniltoa grandiflora	*Pithecellobium dulce*	*Swietenia macrophylla*
Mansoa alliacea	*Plantago major*	*Synedrella nodiflora*
Melaleuca cajuputi	*Plectranthus amboinicus*	*Syngonium podophyllum*
Melia azedarach	*Pluchea indica*	*Syzygium antisepticum*
Mimosa diplotricha	*Plumeria alba*	*Syzygium aqueum*
Mimosa pudica	*Plumeria* sp.	*Syzygium cumini*
Momordica charantia	*Podocarpus* sp.	*Syzygium malaccense*
Montanoa hibiscifolia	*Pogostemon cablin*	*Syzygium oleana*
Morinda citrifolia	*Polyscias scutellaria*	*Syzygium polyanthum*

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TABLE 3. *Continued.*

Tabernaemontana divaricata	*Tilia tomentosa*	*Vigna unguiculata*
Talinum paniculatum	*Tinospora cordifolia*	*Voeniculum vulgare*
Tamarindus indica	*Tithonia diversifolia*	*Wedelia chinensis*
Taraxacum sp.	*Toona sureni*	*Wrightia antidysenterica*
Terminalia catappa	*Tradescantia pallida*	*Zephyranthes candida*
Terminalia mantaly	*Tradescantia spathacea*	*Zingiber cassumunar*
Tevesia burckii	*Tridax procumbens*	*Zingiber officinale*
Theobroma cacao	*Triphasia trifolia*	*Ziziphus spina*
Thunbergia affinis	*Vanilla planifolia*	

FIGURE 4. Collections and extractions during creation of the Indonesia MAGIC Library.

the CMPR website contains a comprehensive list of the collected species, along with all non-sensitive sample information and photographs. The site includes basic search features for easier user navigation. Although specific GPS coordinates are not publicly disclosed to protect plant populations, they can be provided upon request.

A primary goal of this novel resource is to stimulate sustainable and collaborative plant metabolomic and natural products research among scientists in Southeast Asia and globally while decreasing the necessity to export plant materials from the country and allowing Indonesian researchers to remain in full control of their natural resources. Traditional approaches used to build extract libraries often involve destructive and laborious harvesting of large amounts of plant materials, which are then dried in the sun or under hot air before being transported to developed countries for lengthy, multi-step processing and, finally, long-term storage (Eisenberg et al., 2011; Risener et al., 2023). The substantial time necessary to collect, grind, extract and dry plant materials using this traditional approach facilitates the degradation

FIGURE 5. “1st International Conference on Natural Products and Chronic Diseases 2022”. The conference included speakers from Indonesia, Tajikistan, Malaysia, and the USA. The event also featured presentations from graduating CBCD students on their selected topics of research, and a ceremony for the signature of a Memorandum of Understanding between UNAS and Universitas Pakuan.

of unstable phytochemicals, which in turn requires harvesting of additional plant materials and can also lead to the formation of unnatural compounds (Tiwari et al., 2013). The creation of metabolomic libraries using traditional approaches is, therefore, highly laborious, expensive, logistically complicated, and potentially harmful for the affected plant populations. In contrast, the simple method used to establish the Indonesia MAGIC Library is not only highly cost- and time-effective but also sustainable, and it sidesteps the complexities of plant material import/export regulations while mitigating the risk of exploitative bioprospecting.

3.3. Larger context of the project

This project unfolded within the broader objectives of strengthening the partnership between Rutgers and UNAS, expanding the network of scientific collaborations within Indonesia and Southeast Asia, and fostering natural-product-based research, sustainable development and biodiversity conservation. Thanks to generous funding from the USA NIH–FIC and APN, these efforts have achieved significant success.

3.3.1. First international conference and signature of strategic partnership

In June and July 2022, three forums for scientific discussion and networking were held alongside the RAMES-STN workshop described in Section 2.1.1. These included a small conference between UNAS and Rutgers scientists, a formal meeting and discussion between scientists from Rutgers and the Indonesian National Research and Innovation Agency (BRIN) – Indonesia’s government agency in charge of all government affairs in the field of research and technology–, and most notably, a novel International Conference organised by UNAS, the “1st International Conference on Natural Products and Chronic Diseases 2022”, held in Jakarta, Indonesia (iconference-ncd.unas.ac.id). This hybrid conference, focusing on the impact of plant natural products on human health, featured speakers from five universities across four countries and garnered wide coverage from local newspapers. The list of attendees, which included a total of 206 participants, featured representatives from fifteen Indonesian and foreign institutions (Figure 5, Table 4).

TABLE 4. Number of attendees and institutions present at the “1 st International Conference on Natural Products and Chronic Diseases 2022”.

Number of attendees	
Speakers	6
Presenters	16
On-site audience	111
Online audience	58
Other	15
Total	**206**
Number of institutions	
Indonesian	12
Foreign	3
Total	**15**

FIGURE 6. "The International Conference and Workshop in conjunction with the 8th Indonesia Biotechnology Conference 2023". The conference included speakers from BRIN, the Indonesian Ministry of Environment and Forestry, the Indonesian Biotechnology Consortium, Avicenna Tajik State Medical University (Tajikistan), Kyoto University (Japan), Rutgers University (USA), and the private biotechnological sector, among others.

TABLE 5. Number of attendees and institutions present at "The International Conference and Workshop in conjunction with the 8th Indonesia Biotechnology Conference 2023".

Number of attendees	
Speakers	19
Presenters	48
On-site audience	65
Online audience	161
Total	**293**
Number of institutions	
Indonesian	57
Foreign	4
Total	**61**

These discussion forums solidified a strategic partnership among UNAS, BRIN, and Rutgers, leading to the signature of a Memorandum of Understanding (MOU) for long-term collaboration. At the end of 2022, a BRIN researcher and former scientific advisor to the Indonesian president conducted research at the Raskin laboratory at Rutgers as a Fulbright scholar, facilitating further discussion and networking opportunities.

3.3.2. Second international conference and second training workshop

These developments culminated in the rapid organisation of a second, larger international conference as well as a second RAMES-STN training workshop, "The International Conference and Workshop in conjunction with the 8th Indonesia Biotechnology Conference 2023" (https://www.icw-ibc2023.com/). The combined event was jointly organised by BRIN, UNAS, the Indonesian Biotechnology Consortium (KBI), Universitas Mahasaraswati Denpasar (UNMAS) and Rutgers. The two-day hybrid conference took place in Denpasar, Indonesia, in May 2023 and explored the intersection of biodiversity, biotechnology, and health for enhancing sustainable development. It featured nineteen keynote speakers from four countries and attracted 293 participants from 61 institutions (Table 5, Figure 6).

The second RAMES-STN training workshop took place at the Bali Botanical Gardens the day after the conference (Figure 7), following the same full-day format and covering the same topics as the workshop already described in Section 2.1.1. The nineteen participants were conference attendees who had registered for the workshop due to their personal interest, and they included ten researchers, five PhD students, three MSc students and one senior high school student, coming from fourteen institutions across Indonesia (from Java, Sumatra, and Sulawesi), as well as from Okinawa in Japan and from the USA (Figure 2, Table 2). Careful workshop planning prior to the event helped the training run smoothly despite the potential challenges posed once again by the diverse backgrounds of participants, the time limitations and potential language and technical barriers. Once again, the workshop was not formally assessed but rather followed by a post-workshop Q&A session and discussion, during which trainees offered

FIGURE 7. RAMES-STN training workshop at Bali Botanical Gardens in May 2023.

highly positive feedback, expressing eagerness to incorporate the learned techniques into their research. Post-workshop, BRIN representatives received a kit containing all necessary materials for performing RAMES extraction and STN bioassays on 1,000 samples. Both events received thorough coverage from local newspapers as well as the BRIN and KBI websites. Furthermore, two additional Indonesian universities have recently joined the Indonesia MAGIC Library and will participate in future sample collections: Universitas Surabaya (UBAYA) from East Java and Universitas Pancasila from Jakarta.

3.4. Ways forward and areas for future improvement

3.4.1. Suggested next steps

As a completely new resource, the Indonesia MAGIC Library provides numerous avenues for contributing to research, conservation, and economic development in Indonesia. A suggested starting point would be conducting quality control and stability studies of the RAMES disc contents. This can be achieved by eluting the extracts, identifying and quantifying metabolites through UPLC/MS analysis, and comparing them with freshly prepared extracts. A subsequent step could involve rapid functional screening by assessing the antioxidant, antifungal, and antibacterial bioactivities of the extracts with the corresponding STN bioassays.

As Rutgers scientists have developed a broad range of simple, rapid, and portable bioassays beyond the antioxidant, antibacterial, and antifungal – including, for example, assays to detect anti-roundworm, anti-flatworm, anti-Leishmania, or wound healing properties – this rapid functional screening can be broadened to include many bioactivities of interest. Upon identifying extracts with promising bioactivities, it is recommended that detailed metabolic profiling and extensive quantitative analysis of their constituents be performed.

The present pilot version of the library focused on Indonesian plant species with known medicinal properties. Future expansion efforts of the library are also planned to include Indonesian endemic and endangered plant species. In parallel, it would be of great interest to expand the collection by including marine organisms, fungi and insects. Another course of action could be resampling already catalogued species in different seasons or under varied stress conditions to conduct comparative studies, which would provide valuable insights into the impact of environmental and biological stresses on metabolomic profiles. In any case, each new RAMES sample collected in the future should also include a supplemental small, dehydrated piece of tissue (under one gram would suffice) within the same envelope for DNA barcoding and other sequencing strategies, which would serve as a means to validate species identification, thus providing a practical substitute for traditional herbarium vouchers. The incorporation of genomic samples into the Indonesia MAGIC Library was an integral part of the initial vision, as indicated by the acronym MAGIC, which stands for Metabolome And Genome Innovation and Conservation. Consequently, Rutgers scientists have developed and validated a rapid method for field collecting plant samples for DNA studies and barcoding, using silica gel as a drying method (Skubel et al., 2018). In the future, it might also be interesting to develop a near-infrared (NIR) spectroscopy method for the metabolite profiling of RAMES samples, which could serve as a simple, portable, and cost-effective alternative to the current LC-MS method.

Lastly, raising awareness of this unique biological resource at academic gatherings like conferences and scholarly forums is crucial. By doing so, we hope to initiate sample sharing among researchers, leading to the establishment of collaborative research projects within Indonesia and worldwide. Moreover, we expect that awareness of the Indonesia MAGIC Library will encourage more institutions to participate in the collection of samples, thus ensuring its growth over time.

3.4.2. Challenges faced and recommendations for the future

It must also be noted that the project encountered several challenges described below. While these were tackled as effectively as possible, they require attention and strategic planning, providing valuable lessons for any future initiatives. These issues also emphasise the need for subsequent studies to validate the quality of the library.

3.4.2.1. Difficulties in accessing resources

As the UNAS team prepared to conduct functional screening of the samples using STN bioassays, they faced difficulties in procuring the necessary reagents within Indonesia. They encountered significantly higher prices than in the USA and lengthy delivery times of approximately three months. To circumvent this, reagents were purchased by Rutgers and shipped to Indonesia from the USA. However, this process encountered its own setbacks, including significant customs delays and problems with shipping regulations. Therefore, acquiring these reagents from Indonesia requires a substantial budget and meticulous planning to account for extended waiting periods.

Another concern pertains to the long-term preservation of the metabolites in the discs. While stability studies so far have shown that storage at −20 °C causes only relatively minor quantitative and qualitative changes compared to fresh extracts (Skubel et al., 2018), Rutgers scientists suggest −80 °C for optimal longevity of large valuable collections such as the Indonesia MAGIC Library. However, UNAS currently lacks a −80 °C freezer, and thus the library samples are stored at −20 °C. Potential solutions may include securing funds for a −80 °C freezer or transferring the collection to an institution equipped with such facilities. The new strategic partnership between UNAS, BRIN and Rutgers opens doors for finding alternative facilities for the library. The future quality control and stability studies mentioned above will also help determine the long-term effect of storage of the discs at −20 °C versus −80 °C.

3.4.2.2. Technical problems caused by local conditions

The high humidity levels in Indonesia extended the typical sample drying time from 3–8 min to approximately 2 h. This prolonged drying period, coupled with moist conditions, could potentially lead to metabolite degradation. To try to minimise metabolite degradation, the drying step was subsequently always conducted in conditions as dry and as protected from direct sun and heat as field conditions allowed. Additionally, equipment such as the Dremel® cordless rotary tool exhibited faster humidity-related corrosion than usual, even when stored in relatively dry environments such as the laboratory. The importance of carefully drying all Dremel components before storage in their original hermetic box was emphasised, measures which helped slow down the rusting process but did not fully stop it. In the future, common desiccants like silica gel can be used both to expedite drying and to try to maximise the useful life of the equipment.

Power outages also pose potential risks. Presently, the laboratory housing the Indonesia MAGIC Library lacks a backup generator to maintain freezer functionality during power disruptions. Even though this type of incident has not occurred, it is recommended that a contingency plan be established.

Solutions might involve installing a backup generator on the premises or relocating the collection to a facility with backup power. Furthermore, when future funding allows the expansion of the library, producing a larger quantity of discs per sample would enable the creation of two mirror libraries, housed on different premises and potentially in different cities.

3.4.2.3. Challenges in maintaining scientific rigour

While the trainees were selected partly for their English proficiency, some language barriers hindered comprehension of certain details from the RAMES manual or during online progress meetings. This was most noticeable when the Indonesian team enlisted temporary assistance for collection and extraction from UNAS graduate students, who had more limited English skills and had not undergone RAMES training. The English instruction manual proved less effective in these instances, so the trainees orally translated procedures into Indonesian, resulting in some loss of detail. To overcome this challenge in the future, Rutgers scientists are committed to creating bilingual training materials, a task that recent advancements in AI-based translation have greatly simplified.

4. CONCLUSION

The creation of this MAGIC Library positions Indonesia as the world's leader in collecting and cataloguing metabolomic samples of its rare, endangered plants. This project underscores the potential of RAMES technology as a tool for promoting conservation and scientific/economic capacity in biodiversity-rich regions like Indonesia. A single RAMES-STN workshop empowered seventeen young Indonesian scientists with these ethical, low-impact, cost-effective, and portable technologies, and they, in turn, became trainers within their institution, sharing the methodology with peers whenever they required support for project-related collections and extractions. The ripple effect of the project also inspired a second training workshop within less than a year, fostering skills in an additional nineteen early-career scientists spanning diverse institutions within Indonesia and Japan. Given the enthusiasm expressed by attendees, the simplicity of the techniques, the materials left behind and the continuing support from Rutgers researchers, we anticipate these methodologies will continue to expand across the scientific community in Southeast Asia.

In parallel, the creation of the Indonesia MAGIC Library, the first of its kind worldwide, marks a significant stride towards preserving the unique and potential life-saving metabolites contained in Indonesian flora. Despite the challenges faced and the need for subsequent studies to verify the quality of the collection, this project demonstrates the feasibility of developing a substantial collection of extracts with very limited resources and within a very short time frame. Thanks to the ease of sharing RAMES discs, this growing library, now available to the global scientific community, paves the way for extensive collaborative natural products research and sustainable product development. We are optimistic that the success of the project will prompt future funding for expanding the library to incorporate other Indonesian regions and taxonomic groups.

Encouraging international collaboration and knowledge exchange is best exemplified by the long-term strategic partnership formed among UNAS, BRIN, and Rutgers. The network of connections has continued to expand through the multiple formal and informal discussion forums, including two international conferences, which have been inspired by the potential of the Indonesia MAGIC Library. We hope that this fostering of knowledge and resource sharing among scientists and conservation specialists from diverse countries leads to other future partnerships that can impact the region's biodiversity conservation.

Overall, the project's success and enduring legacy lie in empowering young Indonesian scientists with useful scientific tools and skills that boost their sense of ownership and stewardship over Indonesia's biodiversity. As the Indonesia MAGIC Library continues to expand, facilitating collaborative research and illustrating the pharmacological and economic value of the species it contains, we anticipate it can be leveraged by Indonesian science policy advisors to push for stricter protection laws in the country, contributing to global efforts towards sustainable development and biodiversity conservation.

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LIST OF ACRONYMS

APN: Asia-Pacific Network for Global Change Research

BRIN: National Research and Innovation Agency (Badan Riset dan Inovasi Nasional)

CBCD: Center for Botanicals and Chronic Diseases

CMPR: Center for Medicinal Plants Research

KBI: Indonesian Biotechnology Consortium (Konsorsium Bioteknologi Indonesia)

MAGIC: Metabolome And Genome Innovation and Conservation

NIH–FIC: National Institutes of Health – Fogarty International Center

RAMES: RApid Metabolome Extraction and Storage

STN: Screens-To-Nature

UBAYA: Universitas Surabaya

UNAS: Universitas Nasional

UNMAS: Universitas Mahasaraswati Denpasar

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