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Antimycobacterial natural products – an opportunity for the Colombian biodiversity

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ABSTRACT

It is estimated that one-third part of the world population is infected with the tubercle bacillus. While only a small percentage of infected individuals will develop clinical tuberculosis, each year there are approximately eight million new cases and two million deaths. *Mycobacterium tuberculosis* is thus responsible for more human mortality than any other single microbial species. The goals of tuberculosis control are focused to cure active disease, prevent relapse, reduce transmission and avert the emergence of drug-resistance. For over 50 years, natural products have served us well on combating infectious bacteria and fungi. During the 20th century, microbial and plant secondary metabolites have helped to double our life span, reduced pain and suffering, and revolutionized medicine. Colombia is a megadiverse country with enormous potential to offer leads for new antimycobacterial drugs. The principal aim of this article is to show a state of the art on antimycobacterial natural products research in Colombia compared to the rest of the world, in order to develop programs for bioprospecting with a view to determining the biological activity for pharmaceutical and industrial application of natural products in our country.

Key words: Natural products, antimycobacterial activity, Colombia biodiversity

Productos naturales antimicobacterianos: una oportunidad para la biodiversidad colombiana

RESUMEN

Se ha estimado que un tercio de la población mundial está infectado con el bacilo tuberculoso. Aunque un pequeño por-

centaje de los individuos afectados desarrollará clínicamente la enfermedad, cada año esta ocasiona aproximadamente ocho millones de nuevos casos y dos millones de muertes. *Mycobacterium tuberculosis* es el agente infeccioso que produce la mayor mortalidad humana, comparado con cualquier otra especie microbiana. Los objetivos de los distintos programas para el control de la tuberculosis son la cura y diagnóstico de la infección activa, la prevención de recaídas, la reducción de transmisión y evitar la aparición de la resistencia a los medicamentos. Por más de 50 años, los productos naturales han sido útiles en combatir bacterias y hongos patógenos. Durante el siglo 20 los metabolitos secundarios provenientes de plantas y microorganismos revolucionaron la medicina ayudando a controlar el dolor y el sufrimiento en miles de personas. Colombia es un país megabiodiverso, con un enorme potencial para ofrecer moléculas líderes, para el desarrollo de nuevos fármacos anti-tuberculosos. El principal objetivo de este artículo es dar a conocer un estado del arte en la investigación de productos naturales antimicobacterianos en Colombia comparando con otros programas de bioprospección existentes en el mundo y así poder valorar las enormes ventajas que puede tener este recurso inexplorado.

Palabras clave: Productos naturales, actividad antimicobacteriana, biodiversidad colombiana

INTRODUCTION

Human tuberculosis (TB) is a contagious-infectious disease mainly caused by *Mycobacterium tuberculosis*, which is an aerobic pathogenic bacterium that establishes its infection usually in the lungs¹. Pulmonary TB, the most common type of the disease, is usually acquired by inhalation of the bacillus from an infectious patient, subsequently causing irreversible destruction of the lung. About one-third of the world population is currently infected with *M. tuberculosis*; which 10% will develop clinical disease, particularly those who are also infected with human immunodeficiency virus (HIV). The deadly infectious disease, tuberculosis (TB), is the leading cause of death worldwide from a single human pathogen, claiming more adult lives than other deadly diseases such as acquired im-

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munodeficiency syndrome (AIDS), malaria, diarrhea, leprosy and all other tropical diseases combined². The World Health Organization (WHO) estimates that active cases of tuberculosis afflict seven to eight million people annually, and lead up to three million deaths per year³.

Although medical regimens exist for treating tuberculosis, they are far from ideal. Treatment usually involves a combination of drugs—isoniazid (INH) and rifampin, which are given for at least 6 months, and pyrazinamide and ethambutol (or streptomycin), which are used only in the first 2 months of treatment⁴. Because this regimen is extremely difficult to adhere to, WHO recommends a program of directly observed treatment, short-course (DOTS), which involves to health care workers routinely watching patients take their medicine⁴. Only 21 percent of the world's TB patients were treated under DOTS in 1998. Inconsistent or partial treatment leads to the development and spread of drug-resistant strains. There is thus an urgent need for shorter, simpler therapeutic and prophylactic regimens to increase adherence. In addition, new drugs are needed to combat the increasing number of multi-drug-resistant strains (MDR-TB) and extensively drug-resistant (XDR-TB) strains and HIV epidemics, led to an increased need to understand the molecular mechanisms of drug action and drug resistance, which should provide significant insight into the development of new compounds^{1,4}. There are five reasons usually given for needing new tuberculosis drugs: (1) to improve current treatment by shortening the total duration of treatment and/or by providing for more widely spaced intermittent treatment, (2) to improve the treatment of MDR and XDR TB, (3) to provide for more effective treatment of latent tuberculosis infection (LTBI) in programs that are able to implement this practice, (4) side effects, especially hepatotoxicity, are an issue that in some cases, forces an untimely treatment termination, and (5) following the discovery of streptomycin in 1944 by Selman Waksman, a 'golden era' of discovery of anti-tuberculous drugs ensued. However, since the 1960s, there have been few developments in available therapies for the treatment of tuberculosis⁵⁻⁷.

THE IMPORTANCE OF NATURAL PRODUCTS AS SOURCE OF NEW DRUGS

Most collections of natural products start as extracts of fresh or dried material prepared by using various solvents. The extracts are complex mixtures of perhaps several hundred different compounds. Traditional bioassay guided fractionation techniques are generally regarded as being too slow to fit into the pace of high throughput screening: the assays may only be run for a few months in an intensive screening campaign, and the purification of active compounds may not be possible in that timeframe⁸.

Different compounds derived from animals, plants and microbes have been used to treat human disease since the dawn of medicine. The investigation of natural products as source of novel human therapeutics reached its peak in the Western pharmaceutical industry in the period 1970–1980,

which resulted in a pharmaceutical landscape heavily influenced by non-synthetic molecules. Of the 877 small-molecule New Chemical Entities (NCEs) introduced between 1981 and 2002, roughly half (49%) were natural products, semi-synthetic natural product analogues or synthetic compounds based on natural-product⁹. In addition to launched products, at least 70 natural product-related compounds were in clinical trials in 2004¹⁰, and exploration of the bioactivity of natural products continues to provide novel chemical scaffolds for further drug inventions¹¹.

Various reasons have been put forward to explain the success of natural products in drug discovery: their high chemical diversity, the effects of evolutionary pressure to create biologically active molecules, the structural similarity of protein targets across many species, and so on⁸.

Because natural products are a proven template for the development of new scaffolds of drugs¹¹, they have received considerable attention as potential anti-TB agents¹². Antimycobacterial active compounds have been found among many skeleton types, mainly from plants, but also from other organisms such as fungi and marine organisms¹³.

NATURAL PRODUCTS WITH ANTIMYCOBACTERIAL ACTIVITY

Naturally occurring pure compounds as well as extracts from higher and lower forms of plants, microorganisms and marine organisms have indicated that inhibitory activity against *M. tuberculosis* is widespread in nature. Many compounds identified using preliminary functional assays have been provided from researchers interested in phytochemical biodiversity. Usually their potential pharmaceutical worth remains unknown since there is lack of data to show that these compounds are adversely affecting mycobacterial survival mechanisms in humans, or have been derived from medicinal plants¹⁴. A list of natural products with antimycobacterial activity is presented in table 1.

Plants

Plant-based drugs have been used worldwide in traditional medicines for the treatment of various diseases. Approximately 60% of world's populations still rely on medicinal plants for their primary healthcare. Plant species still serves as a rich source of many novel biologically active compounds, as very few plant species have been thoroughly investigated for their medicinal properties¹⁵. Thus, there is renewing interest in phytomedicine during last decade and now days many medicinal plant species are being screened for pharmacological activities¹⁶. There have to be opportunities to investigate ethnobotanical antibacterials to discover new drugs. Before the advent of antibiotic therapy, plants were widely accepted as a resource of antiseptic materials, with fresh plants producing an array of volatile natural products with antibacterial activity¹⁷.

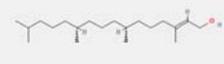
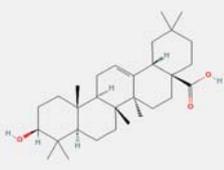
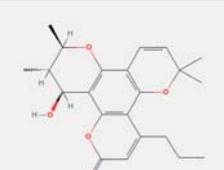
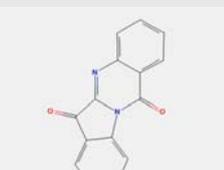
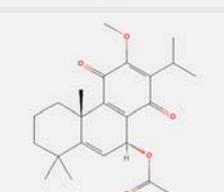
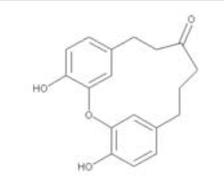
Table 1		Natural products with antimycobacterial activity.		
Source	Compound	Origin		Ref.
Plants	(E)- and (Z)-phytol and phytanol	<i>Leucas volkensii</i>		22
	Oleanolic acid	<i>Lantana hispida</i>		26
	Mulinane	<i>Azorella madreporica</i> Clos		28
	Calanolide A	<i>Calophyllum lanigerum</i>		29
	Tryptanthrin	<i>Strobilanthes cusia</i>		24
	12-methyl-5-dehydroacetylhorninone	<i>Indigofera longeracemosa</i>		30
	(24R)-Saringosterol	<i>Lessonia nigrescens</i>		31
	Engelhardione	<i>Engelhardia roxburghiana</i>		32

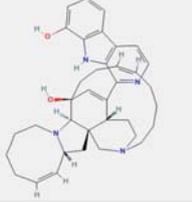
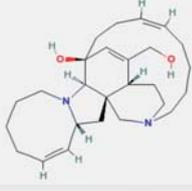
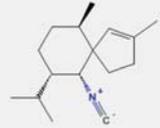
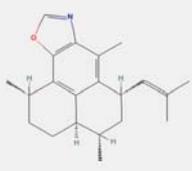
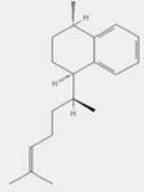
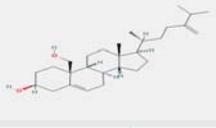
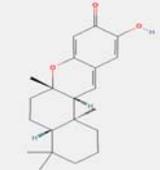
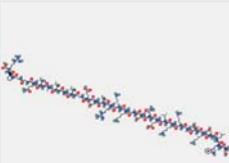
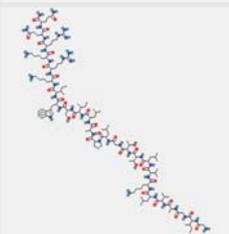
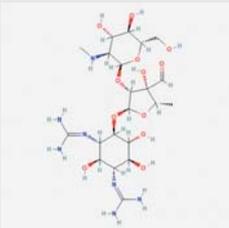
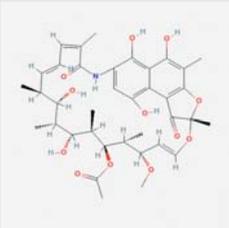
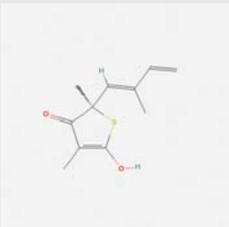
Table 1		Natural products with antimycobacterial activity (cont.).		
Source	Compound	Origin		Ref.
Marine organisms	(+)-8-hydroxymanzamine A	<i>Pachypellina</i> sp		33,34
	Ircinol A	<i>Indo-Pacific sponges</i>		33
	Manzamine A	<i>Indo-Pacific sponges</i>		33,35
	Axisonitrile-3	<i>Acanthella klethra</i>		36
	Pseudopteroxazole	<i>Pseudoptergorgia elisabethae</i>		34
	Erogorgiaene	<i>Pseudoptergorgia elisabethae</i>		37, 38
	Litosterol	<i>Litophyton viridis</i>		39
	Puupehenone	<i>Hytios</i> sp.		40

Table 1		Natural products with antimycobacterial activity (cont.).		
Source	Compound	Origin		Ref.
Insects	Cecropin	<i>Drosophila melanogaster</i>		47
	Melittin	<i>Apis mellifera</i>		47
Micro-organisms	Streptomycin, kanamycin, amikacine and capreomycin	<i>Streptomyces spp</i> , <i>Micromonospora spp</i> , <i>Bacillus spp</i>		49
	Rifamycins	<i>Streptomyces mediterrani</i>		50
	Thiolactomycin	<i>Nocardia sp.</i>		51

Examples of the species which appear to be among the most active include *Allium sativum* (Fam. Liliaceae)¹⁸, *Borrchia frutescens* (Fam. Asteraceae)¹⁹, *Ferula communis* (Fam. Umbelliferae)²⁰, *Heracleum maximum* (Fam. Umbelliferae)²¹, *Karwinskia humboldtiana* (Fam. Rhamnaceae)²¹, *Leucas volkensisii* (Fam. Labiatae)²², *Moneses uniflora* (Fam. Ericaceae)²¹, *Oplopanax horridus* (Fam. Araliaceae)²¹, *Salvia multicaulis* (Fam. Labiatae)²³, *Strobilanthus cusia* (Fam. Acanthaceae)²⁴, *Senna silvestris* (Fam. Leguminosae)²⁵, *Sommeria sabiceoides* (Fam. Rubiaceae)²⁵, *Nectandra hihua* (Fam. Lauraceae)²⁵, *Senna obliqua* (Fam. Leguminosae)²⁵, *Heisteria acuminate* (Fam. Olacaceae)²⁵, *Zanthoxylum sprucei* (Fam. Rutaceae)²⁵, *Lantana*

hispidia (Verbenaceae)²⁶ *Citrus aurantifolia* (Fam. Rutaceae)²⁷, *Citrus sinensis* (Fam. Rutaceae) and *Olea europaea* (Fam. Oleaceae)²⁷.

In some cases, compounds have been isolated which have antimycobacterial activities, for example (E)- and (Z)-phytol and phytanol which were isolated from *Leucas volkensisii*²², pentacyclic triterpenoids from *Lantana hispidia*²⁶.

Other compounds with antitubercular activity from plants are diterpenes as mulinane isolated from *Azorella madreporica* Clos²⁸ and calanolide A produced by a tree native to the tropical rain forest of Sarawak, Malaysia, and it was discovered by

researchers at the National Cancer Institute²⁹. A promising case, exemplified by tryptanthrin, an alkaloid from the Chinese herb *Strobilanthes cusia*. Tryptanthrin and its analogs are potent against multiresistant tuberculosis strains, are non toxic and give promising blood and tissue levels after oral administration to mice²⁴. Furthermore, among the most active natural products, three molecules could be regarded as promising compounds for antitubercular agents from plants: the diterpene 12-methyl-5-dehydroacetylhorninone, isolated from *Indigofera longiracemosa* (Fabaceae)³⁰, the (24*R*)-isomer of the triterpene Saringosterol, obtained from *Lessonia nigrescens* (Lessoniaceae)³¹, and the difenilalkyl ether ketone Engelhardione, isolated from *Engelhardia roxburghiana* (Juglandaceae)³², which were found to inhibit *M. tuberculosis* H37Rv with excellent Minimum Inhibitory Concentration (MIC) values of 0.38, 0.13, and 0.21 mg/L, respectively.

Marine natural products

The oceans are a unique resource that provides a diverse array of natural products, primarily from invertebrates such as sponges, tunicates, bryozoans, and mollusks, and from marine bacteria and cyanobacteria. As infectious diseases evolve and develop resistance to existing pharmaceuticals, the marine environment provides novel leads against fungal, parasitic, bacterial, and viral diseases³³. Although there are a small number of investigators looking at marine products as potential leads against *M. tuberculosis*, the research in this area have been productive³³.

The alkaloid (+)-8-hydroxymanzamine A was firstly isolated from a sponge *Pachypellina* sp and later from an undescribed *Petrosiidae* genus³⁴. This alkaloid exhibits potent inhibitory activity against *M. tuberculosis* H37Rv³⁵. Ircinol A is a manzamine-type alkaloid from Indo-Pacific sponges; this compound represents a useful candidate for assessment *in vivo* against *M. tuberculosis*, since it shows low cytotoxicity and structural complexity compared with the other manzamine-type alkaloids³⁵. In addition, manzamine A inhibits *M. tuberculosis* H37Rv³⁵. Other interesting compound is axisonitrile-3 which is a cyanosquiterpene isolated from the sponge *Acanthella klethra* and shows potent inhibitory activity against *M. tuberculosis*³⁶. Pseudopteroxazole, a benzoxazole diterpene alkaloid isolated from the West Indian gorgonian *Pseudopterogorgia elisabethae*, induces 97% growth inhibition for *M. tuberculosis* H37Rv without substantial toxic effects³⁷. From the hexane extract of the same West Indian gorgonian was isolated erogorgiaene, a serrulatane-based diterpene (also known as biflorane), and induced 96% growth inhibition for *M. tuberculosis* H37Rv³⁸. Litosterol is a C-19 hydroxysteroid isolated from an Okinawan soft coral *Litophyton viridis*. It inhibited 90% of the growth of *M. tuberculosis*³⁹. Puupehenone induced 99% inhibition of *M. tuberculosis* H37Rv growth. The puupehenones are shikimate-sesquiterpene derived metabolites isolated from sponges of the order Verongida and Dictyoceratida, collected from the Hawaiian Islands⁴⁰.

Insects

As strategies in antagonistic relationships, prokaryotic and eukaryotic organisms have developed hundreds of different cytolytic peptides and proteins during their evolution in different phyla of the plant⁴¹ and animal kingdom⁴². Many cytolytic peptides are specific antimicrobially acting peptides that are part of the innate immune system of invertebrates and vertebrates. They serve as primary defense weapons against invading prokaryotic and eukaryotic microorganisms⁴².

Arachnid (spiders and scorpions) venoms contain toxic peptides with a large range (2–12 kDa) of molecular masses, but spider venoms apparently possess a much higher diversity of ion channel and other cell receptor antagonists than scorpion venoms⁴³.

Gene-encoded antimicrobially acting peptides show great variety in amino acid sequence, structure and target specificity. Many of them are cationic, amphipathic peptides with molecular masses lower than 10 kDa and show a higher specificity to prokaryotic than to eukaryotic cells⁴⁴. The main site of antimicrobial activity is the plasma membrane of bacteria and parasitic protozoans. The unstructured antimicrobial peptides are electrically attracted to negatively charged groups of the cell surface, where they adopt an α -helical conformation and accumulate on the membrane. This can result in the formation of transient pores, membrane perturbation and cell lysis⁴⁵. These kinds of compounds could be promising new antitubercular drugs. Some of them, like cecropin and melittin, isolated from different insects, possess antimycobacterial activity^{46, 47}.

Microorganisms

Microorganisms have been exceptionally rich sources of drugs, including antibiotics, immunosuppressants and the lipid-lowering statins. However, these drugs have been produced from a very small range of the world's microbial diversity and how many species of microorganism there might be is not known. Only approximately 6000 bacterial species have been named (compared with more than a million plants and animals) and estimates of 1.5 million species of fungi and 1.5 million species of algae and prokaryote might have to be revised upwards⁴⁸.

Although streptomycin was not the first antibiotic (penicillin, a fungal product, had been isolated some years earlier), its discovery was a landmark in antibiotic history. It was the first effective therapeutic for tuberculosis⁴⁹. Selman Waksman's commitment to the isolation and screening of soil bacteria in the search for bioactive small molecules, especially potential antibiotics, was validated by the discovery of streptomycin⁵⁰.

A variety of bacterial genera have been shown to produce aminoglycoside-aminocyclitol antibiotics. These include *Streptomyces*, *Micromonospora*, *Bacillus*, and so on. Only those compounds emanating from *Streptomyces* are named "-mycins" (e.g., tobramycin) while others are "-micins" (gentam-

icin), "-osins," "-asins," or "-acins." Other aminoglycoside used for tuberculosis therapy are kanamycin, amikacin and capreomycin⁴⁹. Important drugs for TB treatment are rifamycins (RMP) which are a group of semisynthetic antibiotics of rifamycin B, isolated from *Streptomyces mediterranei*⁴⁹.

Other compound from microorganisms is thiolactomycin [(4R)(2E,5E)-2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-iodide] which is a unique thiolactone antibiotic isolated initially from a soil *Nocardia* sp. exhibiting anti-TB activity by inhibiting mycolic acid biosynthesis⁵¹.

ANTIMYCOBACTERIAL NATURAL PRODUCTS: AN OPPORTUNITY FOR THE COLOMBIA BIODIVERSITY

Biological diversity or biodiversity is changing life forms and manifests itself in the genetic diversity of populations, species, ecosystems and landscapes⁵². Colombia has a continental extension of 114'174,800 hectares, which represents about 0.7% of the land surface worldwide. This area is 10% of the world's biodiversity, making Colombia a "megadiverse country"⁵².

The Colombian territorial extension, 53,2 million hectares are covered by natural forests; 21,6 million for other types of vegetation in areas of savannah, dry and wetlands; 1,10 million by inland, snow peaks and settlements urban and at least 38,4 million are under agricultural use and colonization processes. These broad categories of coverage are home to a large ecosystem diversity that is characteristic of Colombia⁵².

The Colombian ecosystem diversity is such that not many ecosystems that exist in the world that are not represented in Colombia⁵².

It has been estimated that Colombia has between 45,000 and 55,000 plant species. This number is very high for a medium-sized country, if we believe that every sub-Saharan Africa contains about 30,000 species, and Brazil, which covers an area 6,5 times greater than that of Colombia, has 55,000⁵².

There are detailed studies of certain groups in marine areas of the Caribbean and less complete in the Pacific coast. According to recent research the total number of species in the reefs is 326, also an estimated number of 700 species (about 200 still not registered) throughout the Caribbean coast of Colombia. Some areas are particularly rich in species, such as the waters of the region of Santa Marta and the Tayrona National Park, where there are nearly 300⁵².

Among insects, some groups stand for diversity and endemism in Colombia. Regarding the butterflies, for example, the group of brown butterflies (Satyridae, tribe Pronophilini) presents its greatest diversity in the Andean countries between Venezuela and Bolivia, preferably in montane habitats between 1,000 and 4,000 meters, with some species lowlands. In Colombia there can be 3,000 species day, seven families, among which are some endemic, such as *Cissia ucumarensis* and *Actinote iguaguensis*. Regarding the family of scorpions, the Sierra Nevada de Santa Marta Mountains and the East can

be considered as an endemic region⁵².

The need for new antitubercular drugs is an urgency, natural products is an important alternative for provide novel chemical scaffolds for further drug inventions. Previously, it was noted that the vast majority of the world's natural compounds have not been tested for biological activity and that several novel sources of biodiversity are potentially available. As reviewed above, there are new sources of novel biodiversity becoming available for screening, and there have been great advances in the technologies available for purifying and identifying natural products. Colombia possesses a broad biodiversity, this situation makes for a promising country for the discovery of drugs derived from natural sources as Colombia should have comprehensive bioprospecting programs backed by the pharmaceutical industry under the protocols that emerged in The Convention on Biological Diversity (CBD 1992). In the literature only there are five reports about antimycobacterial activity of Colombia natural products⁵³⁻⁵⁷.

CONCLUSIONS

Natural products are an important source of antimycobacterial compounds for leads of new drugs. In the past decade there has been renewed attention and interest in the use of traditional medicine globally. In India, 65% of the population in rural areas uses traditional medicine to help meet their primary health care needs^{58,59}. In Chile 71% of the population, and in Colombia 40% of the population, have used such medicine^{58, 59}.

Colombian biodiversity is an enormous opportunity for the researches and government for develops bioprospecting programs with pharmaceutical companies in a balance between those who are the holders of the biodiversity and the Colombian population and those who would potentiate (create value) in that biodiversity for the health and economic benefit of all parties. But despite this enormous potential, at the National Institute of Food and Drug Surveillance (INVIMA), there is a record of only 95 species approved for medicinal use, of which only 11 are native. In this context, it is necessary to carry out activities allow to characterize and develop suitable trades in medicinal and aromatic plants, on the basis of a clear identification of the real market and thus facilitating the monitoring and control of the relevant entities⁵⁸.

The Convention on Biological Diversity (CBD 1992)⁶⁰, which was opened for signature at the Earth Summit in Rio de Janeiro in 1992, seeks to promote the conservation, sustainable use, facilitated access to, and an equitable sharing of the benefits arising out the utilization of genetic resources. As a part of this larger objective, Article 8(j) of the CBD specifically calls upon its members to 'respect, preserve and maintain knowledge, innovations and practices of indigenous and local communities embodying traditional lifestyles relevant for the conservation and sustainable use of biological diversity, and to promote their wider application with the approval and involvement of the holders of such knowledge, innovations and

practices and encourage the sharing of benefits arising from the utilization of such knowledge, innovations and practices'. To date, over 187 countries (with the notable exception of the US) have ratified the CBD⁶⁰. Under this convention Colombia can use the natural products as source of new leads for different diseases with impact in the public health in all world. The Colombian biodiversity is bigger than other tropical countries as Brazil and Costa Rica. The development of the natural products sector represents an opportunity for the improvement of the standard of living of rural communities and for the conservation of zones rich in biodiversity. Colombia's endemic plants are mainly found in the countryside in high biodiversity zones, and there, through the use and trade of plants and the integration of productive chains, rural and indigenous communities could derive important economic benefits. At the economic level, the natural products sector has interesting opportunities in international and regional markets. There is a common interest in the discovery of new products and new uses; and this represents an opportunity for this sector in Colombia.

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