

Review

# Plant natural products research in tuberculosis drug discovery and development: A situation report with focus on Nigerian biodiversity

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Tuberculosis (TB) remains a disease of global importance with approximately two million deaths annually worldwide. Effective treatment of TB has been hampered by the emergence of drug resistant strains of *Mycobacterium tuberculosis*. The global resurgence of TB and the development of multidrug-resistant tuberculosis (MDR TB) and extensively drug-resistant tuberculosis (XDR-TB), call for the development of new anti-tuberculosis drugs to combat this disease. Plant natural products have a proven global history of treating diseases and ailments. This review aims to provide a situation report of on-going global efforts to discover and develop anti-TB drugs from plants, including plants found within Nigeria's rich flora. For two decades, studies on different families and genera of the plant kingdom have shown the great potential of plants as antimycobacterial agents. These motifs, including those from within Nigeria's flora, are discussed. Chemists, biochemists and molecular biologists have also employed technological developments in separation methods, hyphenated techniques, high throughput assays and microarray analysis, to drive the drug discovery process. Nigeria, and indeed, Africa, needs to look inwards to solve the burden of tuberculosis, by tapping on its rich biodiversity, which the continent is endowed with. There is need for the government to be committed and actively fund anti-tuberculosis research.

**Key words:** Plant natural product, antimycobacterial activity, drug discovery, drug development, Nigerian flora, biodiversity.

## INTRODUCTION

Tuberculosis (TB) has continued to be a major health concern all over the world being the leading cause of death from any single infectious agent. It is estimated that

one-third of the world's population is infected with TB (Dye et al., 1999). The World Health Organization (WHO) reported that in 2012, an estimated 8.6 million people

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developed TB and 1.3 million died from the disease (WHO, 2013). Nigeria remains one of twenty-two high TB burden countries around the world. As a measure to tackle this global public health problem, the WHO came up, in 1995, with a standardized control strategy called directly observed treatment short course (DOTS), also endorsed by the International Union against Tuberculosis and Lung Diseases (IUATL), to detect and cure TB (WHO, 1996). Despite the implementation of this strategy, the incidence, prevalence and mortality rates of TB in Africa have continued to be on the increase, and this trend was forecast to continue to 2015 (Dye et al., 2005). Co-infection of HIV with TB has challenged DOTS as a sole TB control strategy for Africa (Corbett et al., 2006; De Cock and Chaisson, 1999). In 2006, the WHO launched the new "Stop TB Strategy", a 10 year plan for the control of TB (WHO, 2006), but the core of the strategy remained DOTS in essence. In Nigeria, the DOTS programme has been implemented in all states and local government areas in the country and 3,000 DOTS centres have been operating across the country since 2006 (Erah and Ojieabu, 2009). Despite these efforts, the programme has not been fully implemented, and moreover, it is beset by incidence of bacterial resistance.

#### **EMERGENCE AND IMPLICATIONS OF DRUG-RESISTANT *M. TUBERCULOSIS***

Inadequate, incomplete, or improperly supervised treatment regimen, wrong prescription, and co-infection with HIV are responsible for the emergence of resistant strains of *Mycobacterium tuberculosis* (MTB) (Corbett et al., 2006). A particularly dangerous form of drug resistant TB is multidrug-resistant TB (MDR-TB), which is defined as a specific form of drug-resistant TB due to a bacillus resistant to at least isoniazid and rifampicin (first line drugs), the two most powerful anti-TB drugs (Smith and Moss, 1994). There have also been reports on the emergence of extensively drug-resistant tuberculosis (XDR-TB), which means resistance to rifampicin and isoniazid and to any fluoroquinolone, as well as to one of three injectable second-line anti-TB drugs, such as capreomycin, kanamycin and amikacin (Centre for Disease Control, 2006). MDR-TB and XDR-TB take longer to treat with second-line drugs, which are mainly bacteriostatic, and have a lower efficacy than first line drugs. Moreover, these second-line drugs are expensive, toxic, difficult to combine with antiretroviral drugs, and are unavailable in most of Africa (Dean et al., 2002; Carroll et al., 2012). With the emergence of these drug-resistant and new strains of TB, adverse side effects of existing

treatments as well as the long treatment regimen, and the influence of HIV, it has become imperative that an urgent need exists for the discovery and development of new anti-TB agents (Newton et al., 2000). It is noteworthy that for the first time in more than Forty years, a new diarylquinoline drug- Bedaquiline, was recently approved by the Food and Drug Administration (FDA), as a component of a combination therapy for the treatment of MDR-TB (FDA, 2012). The understanding of the complete genome of *M. tuberculosis* is quite critical in TB drug discovery as there is a need for target-based discovery of novel anti-TB agents that can act on a site different from the currently known (Pauli et al., 2005; Chhabria et al., 2009).

#### **NATURAL PRODUCTS IN DRUG DISCOVERY AND DEVELOPMENT**

##### **Natural product chemistry and organic synthesis as tools in rational drug design**

Natural products (NPs) have played and continue to play a significant role in the drug discovery process. For a long period of human existence, NPs were the only form of therapy available for use by sick people or to maintain health. Drugs of natural origin have been classified as: i) natural products, ii) products derived semi-synthetically from natural products, and iii) synthetic products based on natural product models (Cragg et al., 1997). Natural product chemistry and organic synthesis are powerful tools for optimising leads and for generating new diversity from natural scaffolds. The amalgamation of both is an important strategy in rational drug design. Statistics from studies carried out by different workers continue to emphasize the potentials and untapped reservoir of molecules with therapeutic interest. Eighty percent of the world's population depends mainly on NPs for their health care and sixty percent of the orthodox drugs currently in use have their origin from NPs (Cragg and Newman, 2005). Evidence of the importance of NPs is provided by the fact that close to half of the bestselling pharmaceuticals in 1991 were either NPs or their derivatives (O'Neill and Lewis, 1993). Newman and co-workers (2003) reported that 61% of the 877 new chemical entities (with low molecular weights) registered as drugs worldwide during the period of 1981-2002 were or have been inspired by NPs. A total of 29 new NPs and NP-derived drugs were introduced in the United States, Europe and Japan between 2000 and 2003 (Burtler, 2004). More recent studies revealed that between 2005 and April 2010, some 19 NP-based drugs were approved for marketing worldwide (Mishra and Tiwari, 2011).

### Natural products as leads in novel and active chemotypes

There is an urgent need to identify novel, active chemotypes as leads for effective drug development, and, as was dramatically illustrated by the discovery of the “wonder” antibiotics of the 1940s and 1950s, nature is the prime source of such lead discoveries. It has however been estimated that only 5 to 15% of the approximately 250 000 species of higher plants have been systematically investigated for the presence of bioactive compounds (Balandrin et al., 1993). Norman Farnsworth stated in his closing remarks in his guest editorial on “An old source for new drugs” in the August 1995 issue of *Pharmaceutical Technology* that “the world of plants represents a virtually untapped reservoir of novel drugs awaiting imaginative and progressive organisations” (Farnsworth, 1995). The success of NPs in drug discovery can be attributed to their high chemical diversity, biochemical specificity, greater number of chiral centres and increased steric complexity than either synthetic drugs or combinatorial libraries, and the effects of evolutionary pressure to create biologically active molecules by interactions with different proteins and biological targets (Wolfender, 2009; Queiroz et al., 2009).

### Natural products and combinatorial chemistry

Despite the successes recorded, there was a global decline of the NP discovery programme by pharmaceutical companies in the 1990s. This was replaced by combinatorial chemistry, which, with the introduction of High throughput screening (HTS), became the preferred choice in drug discovery and development (Lee and Breitenbucher, 2003). This was based on the premise that combinatorial chemistry would generate libraries consisting of millions of compounds, which would be screened by HTS and produce drug leads by sheer number of molecules. In addition, it would also take care of intellectual property (IP) issues generated with NPs. This technology did not prove successful as results from early combinatorial libraries were often disappointing and only few drugs have been discovered by the combination of HTS and combinatorial chemistry (Burtler, 2004; Kingston, 2011). Interest in NPs was therefore renewed, as they have a proven history of providing medicinal agents and more so, they occupy a complimentary region of chemical space as compared with a typical synthetic compound library (Ortholand and Ganesan, 2004). To achieve a well-balanced drug discovery programme, there is no doubt that NPs play a pivotal role and therefore, must be incorporated into the programme.

Combinatorial chemistry has improved over the years and NPs are used as starting templates in the synthesis of combinatorial libraries (Lee and Schneiber, 2001).

## MEDICINAL PLANT RESEARCH AND DRUG DISCOVERY

### Herbal medicine as an ancient practice

The use of plants and plant preparations for the treatment of diseases has been in existence since. Some of the earliest records of usage of plants as drugs are found in *Artharveda*, which is the basis for Ayurvedic medicine in India, dating back to 2000 BCE; the clay tablets in Mesopotamia, dating to 1700 BCE; and the *Eber Papyrus* in Egypt, dating to 1550 BCE (Sneader, 2005). In developing countries particularly in Africa, the population continues to rely on traditional medicine (TM) for their primary healthcare. It is estimated that up to 80% of the populations in Africa, Asia and Latin America depend mainly on TM for their healthcare needs, involving mainly the use of plant extracts (WHO, 2003). These extracts are used as herbal drugs in form of powders, concoctions, ointment, decoctions and infusions. The limitations of these herbal drugs revolve around lack of documentation, lack of standardization and quality control, dosage, and the common tendency to describe diseases and ailments vaguely (Okogun, 2002). In addition, some of these medicinal plants may be of rare existence, and difficult or impossible to grow and propagate. Therefore, it becomes even more challenging to identify and isolate the active principle/s which would be needed for onward synthesis or derivatisation. Due to these challenges experienced with TM, modern science must be applied to the practice to ascertain the efficacy of the plants used. A guided methodological approach to the discovery of plant based drugs, as previously described by Queiroz et al. (2009); Hostettmann et al. (1997), involves the following steps:

1. Taxonomic identification of the plant.
2. Collection and drying of the vegetable material.
3. Extraction of plant materials using different solvents.
4. Fractionation of the extracts.
5. Analysis of extracts and fractions by a combination of chromatographic methods.
6. Purity control of the isolated compounds.
7. Structure elucidation of the constituents by a combination of diverse spectroscopic techniques (UV/VIS, IR spectrophotometry, carbon and proton nuclear magnetic resonance, mass spectrometry, X-ray diffraction) and chemical techniques (hydrolysis, formation of derivatives, degradation reaction etc).

8. Application of *in vitro* and/or *in vivo* screening models.
9. Pharmacological and toxicological assays (pre-clinical assays).

These steps require an inter-disciplinary approach amongst biochemists, ethnobotanists, molecular biologists, organic chemists, pharmacognosists, pharmacologists, taxonomists, and other scientists interested and involved in medicinal plant research. For example, in the drug discovery process, the ethnobotanist and taxonomist collect and identify the plant, respectively, the chemist and biologist work hand in hand to obtain the active principle responsible for a therapeutic activity. It is only after this identification and possible modifications that the drug goes down the chain of pharmacology and series of clinical trials, to establish its efficacy and safety, before it is accepted as a drug. These processes take at least ten years. Plant derived secondary metabolites (phytochemicals) continue to be an important source of new drugs for the following reasons; a) they find direct medicinal application as drug entities, b) they are useful as leads or templates for synthesis or semi-synthesis of structural analogues or derivatives c) they provide inspiration to organic chemists for drug design and total synthesis of new drug entities, d) they serve as biochemical/pharmacological probes and e) they can be used as small-molecule drug precursors which can be converted into the compound of interest by chemical modification (Salim et al., 2008; Harvey, 2008). Some excellent examples of the potentials of plant natural products and their synthetic/ semi-synthetic analogues are presented in Table 1.

### Strategies for plant selection and compound isolation in drug discovery

The choice of plant genera and species for phytochemical and biological studies can be very difficult especially because the number of plants which have not been studied from this point of view is quite enormous. Selection of plants for the purpose of drug discovery can be categorized into four main groups:

1. Ethnomedicine
2. Field observations
3. Chemotaxonomic relationships
4. Random selection

#### **Ethnomedicine/ ethnobotanical survey**

This approach provides a useful way to discovery of new leads in the drug developmental process by relying on

information provided by the traditional medical practitioners (TMPs). Farnsworth et al. (1985) have reported that at least 119 compounds derived from 90 plant species can be considered as important drugs currently in use in one or more countries, with 77% of these being derived from plants used in traditional medicine. In a similar study, Fabricant and Farnsworth (2001) identified 122 compounds from 94 plant species, which are used globally as drugs, with 80% of these having an ethnomedical use identical or related to the current use of the active elements of the plant.

#### **Field observations**

Secondary metabolites are produced as toxic materials for providing defence against predators, as volatile attractants for pollinators, or as colouring agents to attract or warn other species (Dewick, 2009). Environments such as the tropical forests, where the plants have to survive from all year the around attack of a diversity of other plants, bacteria, fungi, viral strains and insects, must be taken into consideration in the search for pharmacologically active natural products.

#### **Chemotaxonomy**

The chemotaxonomical approach in selecting medicinal plants to be studied provides an insight into the chemical composition and eventual isomers and analogues of biologically active compounds in different plant species of the same genus or family. This approach can be fruitful, as some secondary metabolites are found to be specific to a genus or a family. For example, compounds such as iridoids, triterpene acids, chlorogenic acid derivatives and flavonoids have been reported from members of the Rubiaceae family, such as *Adina racemosa*, *Galium verum*, *Galium tortumense*, *Saprosmas cortechinii*, *Morinda citrifolia* and *Asperula arvensis* (Itoh et al., 2003; Demirezer et al., 2006; Guvenalp et al., 2005; Ling et al., 2002). Observation of a biological/ pharmacological activity in a constituent of a species of the genus or family can lead to investigations into other species, to find analogues with similar activity.

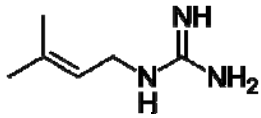
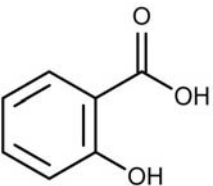
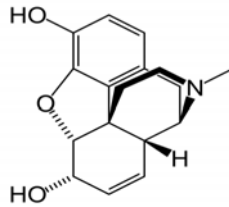
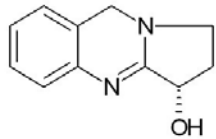
#### **Random selection**

This strategy is especially useful when using high throughput screening techniques where a lot of extracts can be screened at a go. Research institutes such as

**Table 1.** Plant derived drugs of pharmaceutical value.

Botanical source	Isolated compound	Synthetic/semi-synthetic analogue	Therapeutic use
<i>Taxus brevifolia</i> L.	<p>Docetaxel (Taxotere)</p>	Docetaxel (Taxotere)	Anticancer
<i>Podophyllum peltatum</i>	<p>Etoposide</p>	Etoposide	Anticancer
<i>Cannabis sativa</i> L.	<p>Nabilone</p>	Nabilone	Nausea
<i>Artemisia annua</i> L.	<p>Artemisinin</p>	Artesunate, Dihydroartemisinin, Artemeter,	Antimalarial
<i>Amni visnaga</i> (L.) Lam	<p>Khellin</p>	chromolyn sodium	Bronchodilator

Table 1. Contd

<i>Galega officinalis</i> L.		Metformin	Antidiabetic
	Galegine		
Willows bark		Aspirin	Analgesic, antipyretic, anti-inflammatory
	Salicylic acid		
<i>Papaver somniferum</i>		Methadone	Anaesthesia
	Morphine		
<i>Adhatoda vasica</i>		Bromhexine, ambroxol	Mucolytic agent/ bronchodilator
	Vasicine		

National Cancer Research Institute (NCI) at Frederick, Maryland, USA and Central Drug Research Institute, in India, have employed this approach. One of the successful stories from these studies is paclitaxel (Taxol), an anticancer taxane diterpenoid derived from the relatively scarce Pacific or Western yew tree, *Taxus brevifolia* Nutt (Kingston and Newman, 2007).

## BIOLOGICAL EVALUATION OF ANTI-TUBERCULOSIS ACTIVITIES OF PLANT DERIVATIVES

### General aspects of selecting bioassay methods

A major aim of investigations into plants and plant derivatives was to ascertain the biological or pharmacological effects, and this requires suitable bioassays for monitoring these effects. The term 'plant derivatives' is used for crude plant extracts, fractions and compounds, which may be used in modern phytotherapy

(Ameh et al., 2010a, 2010b). In considering the various assay methods available, the guiding factors should be in systems that are simple, rapid, reproducible and inexpensive. For compounds with very low yields, the bioassay has to be sensitive enough for their detection. The number of false positives should also be reduced to a minimum. The complexity of the bioassay has to be designed as a function of the facilities, resources and personnel available. These factors are however determined by the choice of the target organism, depending on its virulence. Developments in automated high throughput screening programs have reduced the time lag experienced with screening of plant extracts. With advances in data handling systems and robotics, a hundred thousand samples can be screened within a week using the 384- well plate (Mishra et al., 2008).

### Test organism

*M. tuberculosis* H<sub>37</sub>Rv available from the American Type

Culture Collection (ATCC 27294) is the organism of choice for antimycobacterial investigations as it has a drug susceptibility profile fairly representative of most drug susceptible clinical isolates. The practicability of working with such a pathogenic organism though, makes this option difficult in many laboratories. This is because there are specific biosafety guidelines that demand the use of laminar-flow hoods and level 3 facility equipment for *M. tuberculosis* laboratorial work. Alternatives to this strain are other slow growing avirulent strains, *M. tuberculosis* H<sub>37</sub>Ra and *M. bovis* BCG which are closely related to *M. tuberculosis* H<sub>37</sub>Rv in terms of genetic composition and drug susceptibility profiles. Many researchers prefer to work with the rapidly growing, avirulent, saprophytic, surrogate *mycobacterium* species which include *Mycobacterium smegmatis*, *Mycobacterium fortuitum* and *Mycobacterium aurum* (McGaw et al., 2008).

### Bioassay guided discovery program

Plants contain a cocktail of many compounds and targeting the bioactive molecule can be a tedious task. To this end, the concept of "bioactivity guided fractionation" was developed to target the isolation of biologically active molecules. Bioassay guided Discovery Program is an inter-disciplinary research between mycobacteriology and natural product chemistry, and this requires a strong collaboration between biologists and chemists. The means of isolation and identification of biologically active compounds from natural sources is referred to as bioassay-guided fractionation (BGF). This methodology involves alternating chromatographic fractionation of extracts and *in vitro* biological testing against a biological target such as the *M. tuberculosis*. Using this method, three potent antimycobacterial compounds were isolated from *Dracaena angustifolia* (Case et al., 2007).

There are advances in fractionation techniques which have been developed towards targeting bioactive compounds in plants. Newer techniques based on liquid-liquid partition, counter-current chromatography (CCC) and centrifugal partition chromatography (CPC), reduce the time-consuming steps experienced with the conventional and older method, adsorption chromatography (Marston and Hostettman, 2006). These techniques have the advantage of loss-free fractionation which is very valuable in bioassay-guided fractionation, as chances of "losing" the anti-TB activity during fractionation are eliminated (Alvi, 2001). There have also been improvements in analytical techniques (fractionation methods hyphenated to spectroscopies or spectrometries such as LC-MS, LC-NMR, LC-UV-DAD) to determine structures. Chemical screening of crude extracts using

hyphenated techniques allows for the efficient targeted isolation of new types of constituents with potential activities as a complimentary approach to bioassay-guided fractionation (Hostettmann, 1997; Wolfender et al., 2001; Queiroz et al., 2009). The hyphenated NMR technique, which is the most powerful of these, has further advanced in the development of new miniaturized probe technologies, with the aim of increasing sensitivity and reducing costs (Hu et al., 2005). Several workers have successfully employed these hyphenated techniques in the identification and isolation of some compounds (Politi et al, 2004; Fu et al., 2010; Lambert et al., 2007). These methods provide some good preliminary information on the nature of constituents of the extract. With this structural information, once the novelty or utility of a given constituent is established, a scale up of the chromatographic process of fraction can then be done to obtain a good yield of the constituent for full structure elucidation as well as biological and pharmacological testing. In this way, isolation of common compounds of little interest (dereplication) is avoided, saving considerable research time.

### Bioassay techniques

Different *in vitro* biological assay methods are used in the biological testing of the efficacy of plant extracts, fractions and compounds against *M. tuberculosis*. These methods include agar disc and well diffusion, micro and macro agar dilution, microbroth dilution, radiorespirometry, reporter gene assays and low oxygen bioassays. Pauli et al. (2005); McGaw et al. (2008) evaluated each of the foregoing methods and discussed their limitations. Results of these bioassays are interpreted as the minimum inhibitory concentration, expressed in terms of µg/mL. Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism (Andrews, 2001). The development of gene expression analysis using microarray technology has led to the sequencing of the *M. tuberculosis* genome in 1998 (Cole et al., 1998). This in turn, has facilitated studies on the mechanism of action of antimycobacterial drugs. Transcriptional profiling of both crude extracts and natural products can provide critical information on both mechanism and detoxification that can be used to guide the drug discovery process (Boshoff et al., 2004). This is demonstrated in the DNA microarray analysis carried out on osthole (7-methoxy-8-isopentenoxycoumarin), a coumarin derivative isolated from many medicinal plants such as *Cnidium monnieri* and *Angelica pubescens*, and which had previously shown antimycobacterial activity. Results show that osthole affected a number of important genes involved in

different metabolic pathways in *M. tuberculosis* (Wei et al., 2013).

## PLANT NATURAL PRODUCTS AS ANTI-TUBERCULOSIS AGENTS

### Worldwide search for plant anti-TB drugs

There has been tremendous research all over the world in the search for novel anti-tuberculosis agents showing that plant derived natural products are potential anti-tuberculosis agents. Research has been performed on screening of plant extracts, based on ethnomedicinal usage, as a preliminary step towards discovering new anti-tuberculosis compounds (Lall and Meyer, 1999; Jimenez-Arellanes et al., 2003; Ibekwe et al., 2012). Despite this, none of the drugs currently used as first or second line drugs in the chemotherapy of tuberculosis has its origin from plant derived natural products. There exist several reports on *in vitro* growth inhibition of different strains of *M. tuberculosis* by plant extracts (Newton et al., 2000; Lall and Meyer, 1999; Gautam et al., 2007; McGaw et al., 2008). Some reviews present the different classes of compounds with antimycobacterial activity. Among these are alkaloids, terpenoids, coumarins/chromones, peptides and phenolics (Okunade et al., 2004; Copp, 2003; Copp and Pearce, 2007; Cantrell et al., 2001). A few of these plant metabolites are shown in Table 2.

Plants belonging to different families and genera have shown antimycobacterial activities. It is noteworthy that most of the plants found to be antimycobacterially active, were ethnomedicinally used for the treatment of tuberculosis or related symptoms such as cough and other respiratory diseases in various societies. Though the compounds responsible for antimycobacterial effects are structurally diverse, they are useful templates for discovery of new pharmaceuticals for the treatment of tuberculosis. Some research has been carried out on structural activity relationships (SAR) by synthesizing derivatives of the parent compounds. This is exemplified in demethoxycurcumin, a compound responsible for the anti-tuberculosis activity of the extracts of *Curcuma longa*.

Semi-synthetic modifications of demethoxycurcumin yielded a novel lipophilic analogue, 4-{4-[7-(3-methoxy-4-methylphenyl)-3,5-dioxohepta-1,6-dienyl]-phenoxy}-but-2-enoic acid ethyl ester, which was 25 times more active at 7.8  $\mu\text{g}/\text{mL}$  than the parent compound (Table 2). The presence or absence of certain functional groups or moieties has been shown to either increase or decrease bioactivity (Agrawal et al., 2008). With the development of new molecular targeted

bioassays such as mycolic acid biosynthesis or cell wall biosynthesis, it will be easier to draw conclusions on structural relationships.

### Some Nigerian plants with antimycobacterial activity

Adeleye and co-workers (2008) evaluated the ethanolic and aqueous extracts of 12 Nigerian medicinal plants for antimycobacterial activity. The study revealed that four of the plant extracts (*Allium cepa*, *Allium ascalonicum*, *Terminalia glaucescens* and *Securidaca longepedunculata*) showed activity on both the culture isolate of *M. tuberculosis* and the control strain (*M. tuberculosis* H<sub>37</sub>Rv) at 0.05 mg/mL. Mann et al. (2008) evaluated some Nigerian medicinal plants for antimycobacterial activity and found four plants giving antimycobacterial activity at  $\leq 1250 \mu\text{g}/\text{mL}$ . These plants were *Anogeissus leiocarpus*, *Terminalia avicennoides*, *Combretum spp.* and *Capparis brassii*. In another related study, eighty-six Nigerian plant based ethnomedicinal remedies were screened for antimycobacterial activity. Sixty nine percent of the extracts showed anti-tuberculosis activity *in vitro*, with 22% revealing activity at  $< 500 \mu\text{g}/\text{mL}$ . Some of the plants which showed promising activity were *Ficus sur*, *Pavetta crassipes*, *Combretum molle*, *Waltheria indica* and *Crotolaria lachnosema*, *Anogiessus leiocarpus*, *Calliandra portoricensis*, *Cassia sieberiana*, *Abrus precatorius* and *Cussonia arborea* (Ibekwe et al., unpublished).

## NIGERIAN BIODIVERSITY AND CHALLENGES IN TB DRUG DISCOVERY

### Nigerian biodiversity, biogeography and anti-TB drug discovery

The biodiversity of the Nigerian flora provides great possibilities in finding novel anti-tuberculosis compounds. Gbile and Adesina (1987), in their review paper, highlighted a good number of Nigerian medicinal plants with biological or therapeutic activities, stressing the pharmaceutical potentials of these plants. Nigeria has a tropical climate with sharp regional variances depending on rainfall. Based on the rainfall distribution, with a wet south and a dry northern half, and also factors such as soil, elevation and human impact on the environment, there are two broad vegetation types; forests and savanna. Nigerian ecology varies from a tropical forest in the south to dry savanna in the far north, yielding a diverse mix of plant and animal life (Microsoft Encarta Encyclopedia, 2006). Every year, millions of square kilometers of biodiversity are lost in Nigeria to the growing herbal market, indiscriminate felling of trees for



**Table 2.** Some plant anti-TB agents in literature.

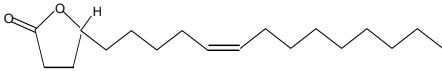
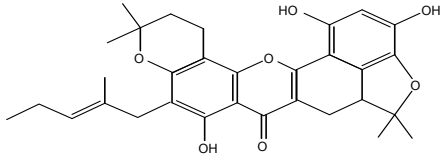
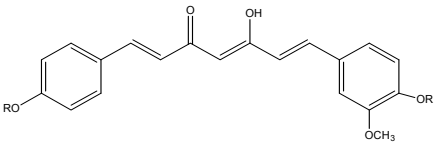
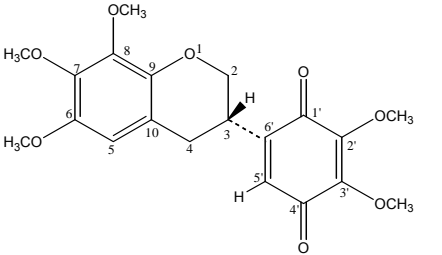
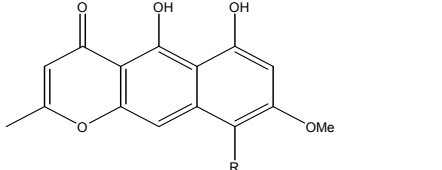
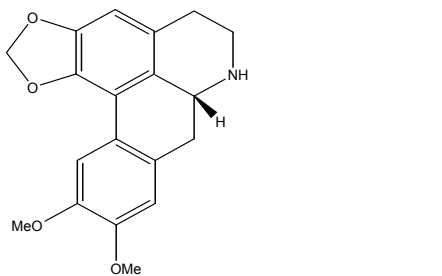
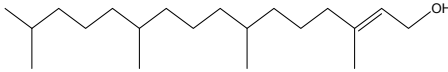
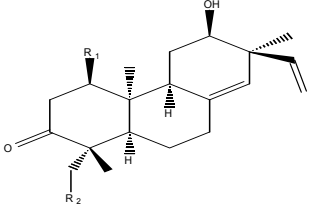
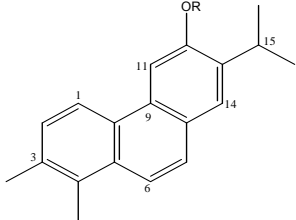
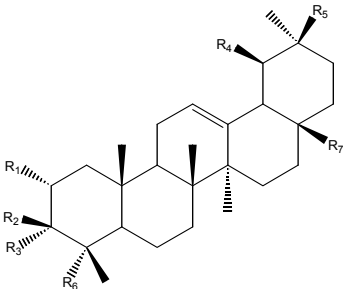
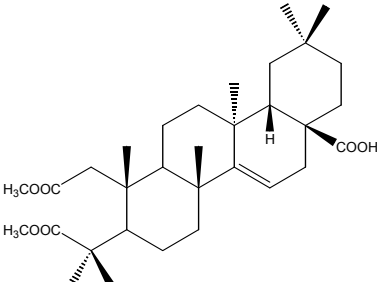
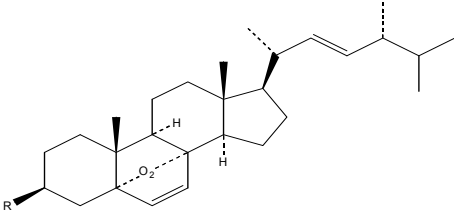
Country	Botanical source	Structure and name of active compound	MIC value (in $\mu\text{g/mL}$ )	Reference
Vietnam	<i>Micromelum hirsutum</i>	 (-)-Z-9-octadecenel-4-olide	5.6	Ma et al., 2005
Thailand	<i>Artocarpus rigidus</i>	 Artonin F	6.25	Namdaung et al., 2006
India	<i>Curcuma longa</i>	 (a) R = H (Demethoxycurcumin) (b) R = $\text{CH}_2\text{-CH=CH-COOCH}_2\text{CH}_3$ (4-{4-[7-(3-methoxy-4-methylphenyl)-3,5-dioxohepta-1,6-dienyl]-phenoxy}-but-2-enoic acid ethyl ester)	(a) 200 (b) 7.8	Agrawal et al., 2008
China	<i>Abrus precatorius</i>	 Abruquinone B	12.5	Limmatvapirat et al., 2004
Ecuador and Peru	<i>Senna oblique</i>	 (a) R = Me (Quinquangulin) (b) R = H (Rubrofasarin)	12	Graham et al., 2004
Thailand	<i>Goniothalamus laoticus</i>	 (-)nordicentrine	12.5	Lekphrom et al., 2009

Table 2. Continue

Taiwan	<i>Leucas volkensii</i> , <i>Morinda citrifolia</i> , <i>Pourthiaea lucida</i>	 (E)-phytol	2-32	Rajab et al., 1998; Saludes et al., 2002; Chen et al., 2010
South America	<i>Sapium haemospermum</i>	 lecheronol A	4	Woldemichael et al., 2004
Middle East	<i>Salvia mauticaulis</i>	 12-demethylmulticauline	0.46	Ulubelen et al., 1997
Latin America, Mexico and South Africa	<i>Junellia tridens</i> , <i>Valeriana laxiflora</i> , <i>Lantana hispida</i> , <i>Buddleja saligna</i>	 Oleanolic acid	5- 50	Caldwell et al., 2000, Gu et al., 2004, Jiménez-Arellanes et al., 2007, Bamuamba et al., 2008
Southeast Asia	<i>Elatoriospermum tapos</i>	 2,3-secotaraxer-14-ene-2,3,28-triic acid 2,3-dimethyl ester	4.13	Pattamadilok and Suttisri, 2008
Kenya	<i>Ajuga remota</i>	 Ergosterol-5,8-endoperoxide	1	Cantrell et al., 1999

domestic uses, bush burning and urbanization. This loss as predicted on a global basis, will lead to a considerable number of species extinction by the year 2050 (Jenkins, 2003). This effect will undermine the drug discovery process, particularly, bio-conservation in general. The convention on biological diversity (CBD), an international treaty which was ratified in 1992, seeks to promote biodiversity conservation, sustainable use of biodiversity, and equitable sharing of benefits from the use of genetic resources. Though the CBD presents some limitations, especially from the legal angle, as exemplified in a study carried out in Suriname, the overall objective of the treaty should be applauded and abided by (Kingston, 2011).

Nigeria, as a country endemic with tuberculosis, has at its disposal, a huge biodiversity of higher plants and an increasing number of scientists to carry out the needed studies to find plant extracts and compounds active against TB (Cadmus et al., 2010). The country also has a rich culture and history of ethnomedicine. A variety of plants are used locally in the treatment of tuberculosis, but have not been investigated for their anti-tuberculosis properties. These plants provide a rich variety of isolatable phytochemicals, but due to lack of infrastructure and adequate scientific facilities to carry out research, Nigeria and indeed, Africa continues to depend on the Western countries for the discovery of new drugs. Considering the high prevalence of tuberculosis in Africa, it has become imperative for African governments to fund anti-TB drug research and develop capacities more aggressively. The government, pharmaceutical companies and health related non-governmental organizations such as the African Network for Drug and Diagnostic innovation (ANDi) can collaborate in a public private partnership (PPP) scheme to robustly fund research in this area, thus using the biodiversity of the country to address the health needs of the nation, and ultimately promote economic development.

## DEDUCTIONS AND CONCLUSIONS

The following deductions and conclusions are evident from the foregoing:

1. Plants are an invaluable source for discovering potentially new antimycobacterial compounds. Positive correlation exists between antimycobacterial activity results on Nigerian plants and ethnomedical/traditional usage.

There is a need for further studies on the correlation between structural features and anti-tuberculosis activity as some bioactive phytochemicals which have proved useful as model compounds or templates can be

employed in the synthesis or semi-synthesis of new drugs.

2. Further investigations are needed for the development of new anti-tuberculosis drugs, which include both functional (*in vivo* assays) and mechanistic (micro-array assays) studies. In the future, advances in the understanding of immunology and related areas should permit the development of new selective and sensitive bioassays to guide the isolation of bioactive natural products.

3. If the current trends of destruction of tropical forest habitats and general global simplification of the biota continue at their present rates, biochemists, ethnobotanists, molecular biologists, organic chemists, pharmacognosists, pharmacologists, taxonomists, and other scientists interested and involved in medicinal plant research may have only a few decades remaining to survey and sample the diverse chemical constituents of the plant kingdom for potentially useful novel bioactive compounds. It is imperative that endangered, fragile, and over-exploited genetic resources would be preserved to the greatest extent possible for future generations who may possess the tools (both technical and intellectual) necessary to successfully exploit and manage these resources more intelligently.

4. The government of Nigeria, and indeed, Africa, has a key role to play in the funding of anti-tuberculosis research. With non-profit organisations like ANDi, which is committed to better health care delivery for Africans, by Africans, government can redeem its pledge of the signatories of the 2000 Abuja declaration to increase support for research (including operational research) to develop new tools and improve existing ones.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

## REFERENCES

- Adeleye IA, Onubogu CC, Ayolabi CI, Isawumi AO, Nshiogu ME (2008). Screening of crude extracts of twelve medicinal plants and "wonder-cure" concoction used in Nigeria unorthodox medicine for activity against *Mycobacterium tuberculosis* isolated from tuberculosis patients sputum. *Afr. J. Biotechnol.* 7(18):3182-3187.
- Agrawal DK, Saikai D, Tiwari R, Ojha S, Shanker K, Kumar JK, Gupta AK, Tandon S, Negi, AS, Khanuja SPS (2008). Demethoxycurcumin and its semisynthetic analogues as antitubercular agents. *Planta Med.* 74:1828-1831.
- Alvi KA (2001). Screening natural products: bioassay-directed isolation of active components by dual-mode CCC. *J. Chromatogr. Rel. Tech.* 24:1765-1773.
- Ameh SJ, Obodozie OO, Abubakar MS, Garba M (2010a). Current phytotherapy - a perspective on the science and regulation of herbal

- medicine. *J. Med. Plant Res.* 4(2):72-81.
- Ameh SJ, Obodozie OO, Abubakar MS, Garba M (2010b). Current phytotherapy – an inter-regional perspective on policy, research and development of herbal medicine. *J. Med. Plant Res.* 4(15):1508-1516.
- Andrews JM (2001). Determination of minimum inhibitory concentrations. *J. Antimicrob Chemoth.* 48:5-16.
- Balandrin MF, Kinghorn AD, Farnsworth NR (1993). Plant derived natural products in drug discovery and development: an overview. In Kinghorn AD, Balandrin MF (eds). *Human Medicinal Agents from Plants*. ACS symposium series 534. Washington D.C: Am. Chem. Soc. pp. 2-12.
- Bamuamba K, Gammon DW, Meyers P, Dijoux-Franca MG, Scott G (2008). Anti-mycobacterial activity of five plant species used as traditional medicines in the Western Cape Province (South Africa). *J. Ethnopharmacol.* 117(2):385-390.
- Boshoff HIM, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry CE III (2004). The transcriptional responses of *Mycobacterium tuberculosis* to inhibitors of metabolism. *J. Biol. Chem.* 279:40174-40184.
- Burtler MS (2004). The role of natural product chemistry in drug discovery. *J. Nat. Prod.* 67:2141-2153.
- Cadmus SI, Okoje VN, Taiwo BO, Soolingen DV (2010). Exposure of dentists to *Mycobacterium tuberculosis*, Ibadan, Nigeria. *Emerg. Inf. Dis.* 16 (9):1479-1481.
- Caldwell CG, Franzblau SG, Suarez E, Timmermann BN (2000). Oleanane triterpenes from *Junellia tridens*. *J. Nat. Prod.* 63(12):1611-1614.
- Cantrell CL, Franzblau SG, Fischer NH (2001). Antimycobacterial plant terpenoids. *Planta Med.* 67(8):685-694.
- Cantrell CL, Rajab MS, Franzblau SG, Fronczek FR, Fischer NH (1999). Antimycobacterial ergosterol-5,8-endoperoxide from *Ajuga remota*. *Planta Med.* 65(8):732-734.
- Carroll MW, Lee M, Cai Y, Hallahan CW, Shaw PA, Min JH, Goldfeder LC, Alekseyev V, Grinkrug S, Kang HS, Hwang S, Park HM, Kang E, Lee SY, Jin B, Park HE, Min S, Park SK, Jeon DS, Via LE, Barry CE III (2012). Frequency of adverse reactions to first- and second-line anti-tuberculosis chemotherapy in a Korean cohort. *Int. J. Tuberc. Lung D.* 16:961-966.
- Case RJ, Wang Y, Franzblau SG, Soejarto DD, Maitainaho L, Piskaut P, Pauli GF (2007). Advanced applications of counter-current chromatography in the isolation of antituberculosis constituents from *Dracaena angustifolia*. *J. Chromatogr. A.* 1151:169-174.
- Centre for Disease Control (2006). Emergence of *Mycobacterium tuberculosis* with extensive resistance to second-line drugs worldwide, 2000-2004. Retrieved Nov 2006 from <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5511a2.htm>
- Chen J, Lin W, Shieh P, Chen I, Peng C, Sung P (2010). A new long chain alkene and antituberculosis constituents from the leaves of *Pourthiaea lucida*. *Chem. Bio. Divers.* 7:717-721.
- Chhabria M, Jani M, Patel S (2009). New frontiers in the therapy of tuberculosis: fighting with the global menace. *Mini-Rev. Med. Chem.* 9:401-430.
- Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nat.* 393(6685):537-544.
- Copp BR (2003). Antimycobacterial natural products. *Nat. Prod. Rep.* 20:535-557.
- Copp BR, Norrie Pearce A (2007). Natural product growth inhibitors of *Mycobacterium tuberculosis*. *Nat. Prod. Rep.* 24:278-297.
- Corbett EL, Marston B, Churchyard GJ, De Cock KM (2006). Tuberculosis in sub-Saharan Africa: opportunities, challenges, and change in the era of antiretroviral treatment. *Lancet* 367:926-937.
- Cragg GM, Newman DJ (2005). Biodiversity; continuing sources of novel drug leads. *Pure Appl. Chem.* 77:7-24.
- Cragg GM, Newman DJ, Snader, KM (1997). Natural products in drug discovery and development. *J. Nat. Prod.* 60:52-60.
- De Cock KM, Chaisson RE (1999). Will DOTS do it? A reappraisal of tuberculosis control in countries with high rates of HIV infection. *Int. J. Tuberc. Lung D.* 3:457-465.
- Dean GL, Edwards SG, Ives NJ (2002). Treatment of tuberculosis in HIV-infected persons in the era of highly active antiretroviral therapy. *AIDS* 16:75-83.
- Demirezer LO, Gurbuz F, Guvenalp Z, Stroch K, Zeeck A (2006). Iridoids, flavonoids and monoterpene glycosides from *Galium verum* subsp. verum. *Turk. J. Chem.* 30:525-534.
- Dewick PM (2009). *Medicinal natural products: A biosynthetic approach*. 3<sup>rd</sup> ed. United Kingdom: Wiley. 550 P
- Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC (1999). Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. *JAMA* 282:677-686.
- Dye C, Watt CJ, Bleed DM, Hosseini SM, Raviglione MC (2005). Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. *JAMA* 293(22):2767-2775.
- Erah PO, Ojieabu WA (2009). Success of the control of Tuberculosis in Nigeria: A review. *Int. J. Health Res.* 2(1):3-14.
- Fabricant DS, Farnsworth NR (2001). The value of plants used in traditional medicine for drug discovery. *Environ. Health Persp.* 109:69-75.
- Farnsworth NR (1995). An old source for new drugs. *Pharm. Tech.* Aug: 14-15.
- Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z (1985). Medicinal plants in therapy. *B. World Health Organ.* 63(6):965-981.
- Food and Drug Administration (2012). FDA News Release. Retrieved March 2013 from <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm333695.htm>.
- Fu S, Arráez-Roman D, Segura-Carretero A, Menéndez JA, Menéndez-Gutiérrez MP, Micol V, Fernández-Gutiérrez A (2010). Qualitative screening of phenolic compounds in olive leaf extracts by hyphenated liquid chromatography and preliminary evaluation of cytotoxic activity against human breast cancer cells. *Anal. Bioanal. Chem.* 397:643-654.
- Gautam R, Saklani A, Jachak SM (2007). Indian medicinal plants as a source of antimycobacterial agents. *J. Ethnopharmacol.* 110(2):200-234.
- Gbile ZO, Adesina SK (1987). Nigerian flora and its pharmaceutical potential. *J. Ethnopharmacol.* 19:1-16.
- Graham JG, Zhang H, Pendland SL, Santarsiero BD, Mesecar AD, Cabieses F, Farnsworth NR (2004). Antimycobacterial naphthopyrones from *Senna obliqua*. *J. Nat. Prod.* 67:225-227.
- Gu JQ, Wang Y, Franzblau SG, Montenegro G, Yang D, Timmermann BN (2004). Antitubercular constituents of *Valeriana laxiflora*. *Planta Med.* 70:509-514.
- Guvenalp Z, Demirezer LO (2005). Flavonol glycosides from *Asperula arvensis* L. *Turk. J. Chem.* 29:163-169.
- Harvey AL (2008). Natural products in drug discovery. *Drug Discov. Today* 13:894-901.
- Hostettmann K, Wolfender JL, Rodriguez S (1997). Rapid detection and subsequent isolation of bioactive constituents of crude plant extracts. *Planta Med.* 63:2-10.
- Hu JF, Garo E, Yoo HD, Cremin PA, Zeng L, Goering MG (2005). Application of capillary-scale NMR for the structure determination of phytochemicals. *Phytochem. Anal.* 16:127-133.
- Ibekwe NN, Orishadipe AT, Boshoff H, Adesomoju AA, Okogun JI,

- Barry CE (2012). *In vitro* antimycobacterial studies on the leaf extracts and fractions of *Pavetta crassipes* K. Schum. Afr. J. Pure. App. Chem. 6(2):55-58.
- Itoh A, Fujii K, Tomatsu S, Takao C, Tanahashi T, Nagakura N, Chen C (2003). Six secoridoid glucosides from *Adina racemosa*. J. Nat. Prod. 66:1212-1216.
- Jenkins M (2003). Prospects for biodiversity. Sci. 302:1175-1177.
- Jimenez-Arellanes A, Meckes M, Ramirez R, Torres J, Herrera-Luna J (2003). Activity against multidrug-resistant *Mycobacterium tuberculosis* in Mexican plants used to treat respiratory diseases. Phytother. Res. 17:903-908.
- Jiménez-Arellanes A, Meckes M, Torres J, Luna-Herrera J (2007). Antimycobacterial triterpenoids from *Lantana hispida* (Verbenaceae). J. Ethnopharmacol. 111:202-205.
- Kingston DG, Newman DJ (2007). Taxoids: cancer-fighting compounds from nature. Curr. Opin. Drug Disc. 10:130-144.
- Kingston DGI (2011). Modern Natural Products Drug Discovery and its relevance to biodiversity conservation. J. Nat. Prod. 74: 496-511.
- Lall N, Meyer JJM (1999). *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. J. Ethnopharmacol. 66:347-354.
- Lambert W, Wolfender J-L, Stärk D, Christensen SB, Hostettmann K, Jaroszewski JW (2007). Identification of natural products using HPLC-SPE combined with CapNMR. Anal. Chem. 79:727-735.
- Lee A, Breitenbucher JG (2003). The impact of combinatorial chemistry on drug discovery. Curr. Opin. Drug Disc. 6:494-508.
- Lee ML, Shneider G (2001). Scaffold architecture and pharmacophoric properties of natural products and trade drugs: application in the design of natural product-based combinatorial libraries. J. Comb. Chem. 3:284-289.
- Lekphrom R, Kanokmedhakul S, Kanokmedhakul K (2009). Bioactive styryllactones and alkaloids from flowers of *Goniothalamus laoticus*. J. Ethnopharmacol. 125:47-50.
- Limmatvapirat C, Sirisopanaporn S, Kittakoop P (2004). Antitubercular and antiplasmodial constituents of *Abrus precatorius*. Planta Med. 70:276-278.
- Ling S-K, Morrita A, Tanaka T, Fujioka T, Mihashi K, Kouno I (2002). Iridoids and anthroquinones from the Malaysian medicinal plant, *Saprosma scortechinii* (Rubiaceae). Chem. Pharm. Bull. 50(8):1035-1040.
- Ma C, Case RJ, Wang Y, Zhang HJ, Tan GT, Hung NV, Cuong NM, Franzblau SG, Soejarto DD, Fong HH, Pauli GF (2005). Antituberculosis constituents from the stem bark of *Micromelum hirsutum*. Planta Med. 71:261-267.
- Mann A, Amupitan JO, Oyewale AO, Okogun JI, Ibrahim K, Oladosu P, Lawson L, Olajide I, Nnamdi A (2008). Evaluation of *in vitro* antimycobacterial activity of Nigerian plants used for treatment of respiratory diseases. Afr. J. Biotech. 7(11):1630-1636.
- Marston A, Hostettman K (2006). Developments in the application of counter-current chromatography to plant analysis. J. Chromatogr. A. 1112:181-194.
- McGaw LJ, Lall N, Meyer JJM, Eloff JN (2008). The potential of South African plants against *Mycobacterium* infections. J. Ethnopharmacol. 119:482-500.
- Microsoft Encarta Encyclopedia (2006). Nigeria. Retrieved November, 2006 from <http://encarta.msn.com/encnet/refpages/RefArticle.aspx?refid=761557915>
- Mishra BB, Tiwari VK (2011). Natural products in drug discovery: clinical evaluations and investigations. In Opportunity, challenge and scope of natural products in medicinal chemistry. Research signpost, pp. 1-62.
- Mishra KP, Ganju L, Sairam M, Banerjee PK, Sawhney RC (2008). A review of high throughput technology for the screening of natural products. Biomed. Pharmacother. 62:94-98.
- Namdaung U, Aroonrerk N, Suksamran S, Danwisetkanjana K, Saenboonrueng J, Arjchomphu W, Suksamran A (2006). Bioactive constituents of the root bark of *Artocarpus rigidus* subsp. *rigidus*. Chem. Pharm. Bull. 54(10):1433-1436.
- Newman DJ, Cragg GM, Snader KM (2003). Natural products as sources of new drugs over the period 1981- 2002. J. Nat. Prod. 66:1022-1037.
- Newton SM, Lau C, Wright CW (2000). A review of antimycobacterial natural products. Phytother. Res. 14:303-322.
- O'Neill MJ, Lewis, JA (1993). The renaissance of plant research in the pharmaceutical industry. In Kinghorn AD, Balandrin MF (eds). Human Medicinal Agents from Plants. ACS symposium series 534. Washington D.C: Am. Chem. Soc. pp. 48-55.
- Okogun JI (2002). Drug discovery through ethnobotany in Nigeria: some results. In Iwu MM, Wootton JC (eds). Ethnomedicine and Drug Discovery. Elsevier Science B.V. Amsterdam, The Netherlands, pp. 148
- Okunade L, Elvin-Lewis MP, Lewis WH (2004). Natural antimycobacterial metabolites: current status. Phytochemistry 65:1017-1032.
- Ortholand JY, Ganesan A (2004). Natural products and combinatorial chemistry: back to the future. Curr. Opin. Chem. Biol. 8:271-280.
- Pattamadilok D, Suttisri R (2008). Seco-terpenoids and other constituents from *Elateriospermum tapos*. J. Nat. Prod. 71:292-294.
- Pauli GF, Case RJ, Inui T, Wang Y, Cho S, Fischer NH, Franzblau SG (2005). New perspectives on natural products in TB drug research. Life Sci. 78:485-494.
- Politi M, Sanogo R, Ndjoko K, Guilet D, Wolfender J-L, Hostettmann K, Morelli I (2004). HPLC-UV/PAD and HPLC-MS<sup>n</sup> analyses of leaf and root extracts of *Vismia guineensis* and isolation and identification of two new bianthrone. Phytochem. Anal. 15:355-364.
- Queiroz EF, Wolfender J-L, Hostettmann K (2009). Modern approaches in the search for new lead antiparasitic compounds from higher plants. Curr. Drug Targets 10:202- 211.
- Rajab MS, Cantrell CL, Franzblau SG, Fischer NH (1998). Antimycobacterial activity of (E)-phytol and derivatives: a preliminary structure-activity study. Planta. Med. 64:2-4.
- Salim AA, Chin YW, Kinghorn AD (2008). Drug discovery from plants. In Ramawat KG, Merillon JM (eds). Bioactive molecules and medicinal plants. Springer, pp. 1-24.
- Saludes JP, Garson MJ, Franzblau SG, Aguinaldo AM (2002). Antitubercular constituents from the hexane fraction of *Morinda citrifolia* Linn.(Rubiaceae). Phytother. Res. 16:683-685.
- Smith PG, Moss AR (1994). Epidemiology of Tuberculosis. In Bloom BR (ed). Pathogenesis, Protection and Control. Washington D.C: Am. Soc. Microb. pp. 47-60.
- Snead W (2005). Drug Discovery: a History. Wiley, Chichester, United Kingdom.
- Ulubelen A, Topcu G, Johansson CB (1997). Norditerpenoids and diterpenoids from *Salvia multicaulis* with antituberculous activity. J. Nat. Prod. 60:1275-1280.
- Wei J, Guo N, Liang J, Yuan P, Shi Q, Tang X, Yu L (2013). DNA microarray gene expression profile of *Mycobacterium tuberculosis* when exposed to Osthole. Pol. J. Microbiol. 62:23-30.
- Woldemichael GM, Gutierrez-Lugo MT, Franzblau SG, Wang Y, Suarez E, Timmermann BN (2004). *Mycobacterium tuberculosis* growth inhibition by constituents of *Sapium haematospermum*. J. Nat. Prod. 67(4):598-603.
- Wolfender J-L (2009). HPLC in natural product analysis: The detection issue. Planta Med. 75:719-734.
- Wolfender J-L, Ndjoko K, Hostettmann K (2001). The potential of LC-NMR in phytochemical analysis. Phytochem. Anal. 12:2-22.
- World Health Organisation (1996). Tuberculosis: Directly observed treatment short course. Retrieved November, 2006 from <http://www.who.int/tb/dots/dotsplus/fag/en/index.html>.
- World Health Organisation (2010). Tuberculosis Retrieved November 2006 from

<http://www.who.int/mediacentre/factsheets/fs104/en/print.html>  
World Health Organisation (2013). Global tuberculosis report. Retrieved  
November 2013 from  
[http://www.who.int/tb/publications/global\\_report/](http://www.who.int/tb/publications/global_report/)  
World Health Organization (2003). Traditional Medicine. Retrieved June  
2013 from <http://www.who.int/mediacentre/factsheets/fs134/en/>