



Three promising antimycobacterial medicinal plants reviewed as potential sources of drug hit candidates against multidrug-resistant tuberculosis

Naasson Tuyiringire^{a,b,*}, Serawit Deyno^{a,c}, Anke Weisheit^a, Casim Umba Tolo^a,
Deusdedit Tusubira^{a,d}, Jean-Pierre Munyampundu^e, Patrick Engeu Ogwang^a,
Claude Mambo Muvunyi^f, Yvan Vander Heyden^g

^a Pharm-BioTechnology and Traditional Medicine Centre (PHARMBIOTRAC), Mbarara University of Science & Technology, P. O. Box, 1410, Mbarara, Uganda

^b School of Nursing and Midwifery, College of Medicine and Health Sciences, University of Rwanda, University Avenue, P.O. Box 56, Butare, Rwanda

^c Faculty of Medicine, College of Medicine and Health Sciences, Hawassa University, P. O. Box 1560 Hawassa, Ethiopia

^d Department of Biochemistry, Mbarara University of Science and Technology, P. O. Box, 1410, Mbarara, Uganda

^e School of Science, College of Science and Technology, University of Rwanda, Avenue de l'Armée P.O.Box 3900, Kigali, Rwanda

^f College of Medicine and Health Sciences, University of Rwanda, University Avenue, P.O. Box 56, Butare, Rwanda

^g Department of Analytical Chemistry, Applied Chemometrics and Molecular Modelling, Vrije Universiteit Brussel (VUB), Laarbeeklaan 103 B-1090, Brussels, Belgium

ARTICLE INFO

Keywords:

Multidrug-resistant tuberculosis
Zanthoxylum leprieurii
Lantana camara
Cryptolepis sanguinolenta
Metabolic fingerprinting
Antimycobacterial activity

ABSTRACT

Regimens of current drugs for tuberculosis are lengthy and are associated with many adverse effects. Currently, the emergence of different resistant strains has been observed. This urges a need for the discovery and development of novel drugs. The main sources of drug lead candidates are based on natural products. *Zanthoxylum leprieurii*, *Lantana camara*, and *Cryptolepis Sanguinolenta* are among the plants that have antimycobacterial activity. Recent technological methods, such as metabolomics, can rapidly detect and identify active compounds from medicinal plants. In this review, we aim to provide an overview and discussion of the antimycobacterial activity, phytochemical analysis and toxicity profile of these plants and their products as well as the potential of metabolomic fingerprinting of medicinal plants with a given activity on microbes, in the search for the potential drug hit molecules.

The information for this review was extracted from databases such as Excerpta Medica Database, Google Scholar, Springer, and PubMed Central. Primary studies, using a combination of the keywords antimycobacterial medicinal plant, multidrug-resistant tuberculosis, phytochemistry, toxicity, *Zanthoxylum leprieurii*, *Lantana camara*, *Cryptolepis sanguinolenta*, and plant metabolomics/metabolic fingerprinting of plant extracts, have been considered.

The above-mentioned plant species showed antimycobacterial activity against drug-resistant strains of *M. tuberculosis*. They may provide potential candidates for novel drugs against multidrug-resistant tuberculosis. However, extensive work is still needed. To our knowledge, there is no or limited literature that reports the metabolic fingerprints of these plants. The analysis of the metabolite fingerprints of medicinal plants with similar antimicrobial activity could be important to determine whether the activity results from common metabolites within different plant species. This review shows that these plants are potential candidates to provide drug hits against multidrug-resistant tuberculosis strains. Future studies of compound optimization, *in vivo* safety and efficacy, as well as of the specific mechanisms of action are however required.

1. Introduction

Tuberculosis (TB), a bacterial disease caused by *Mycobacterium tuberculosis* (*M. tb*), continues to harm humans. Approximately 10.0 million people were infected with TB in 2018 and 1.2 million among the

HIV-negative patients were reported dead from the disease. In addition to this, 251,000 HIV-positive persons died from TB [1]. Over 40% of the HIV death rates in 2016 were due to TB [2]. According to WHO [2], over 95% of TB death rates occurred in low- and middle-income countries. In 2016, an estimated 1 million cases of TB in children were reported, of

* Corresponding author. P. O. Box 1410, Mbarara, Uganda.

E-mail addresses: ntuyiringire@std.must.ac.ug, n.tuyiringire@ur.ac.rw (N. Tuyiringire).

<https://doi.org/10.1016/j.tube.2020.101987>

Received 28 April 2020; Received in revised form 13 August 2020; Accepted 13 August 2020

Available online 16 August 2020

1472-9792/© 2020 Elsevier Ltd. All rights reserved.

which 250,000 died (including children with HIV-associated TB). Although active infection with TB is symptomatic and can be treated [3], one-third of the world population is latently infected [4]. Latent infection is asymptomatic and therefore difficult to treat. Current anti-TB regimens are not only lengthy but are also associated with severe adverse effects, such as skin rash, hepatitis, abdominal pain, hypersensitivity reactions, vomiting, headache, and convulsions [5–8]. These drug regimens are also expensive [9–12], and *M. tuberculosis* shows drug resistance to most of the standard anti-TB drugs (multidrug resistance) [7,13,14]. Antibiotic therapy has two possible outcomes for pathogens, which are clearance or failure [15]. If infected patients transmit resistant microbes before clearance/death, selection for resistance occurs [15]. Multidrug-resistant TB (MDR-TB) remains a public health security threat. WHO [2] estimated 600,000 new cases with resistance to the most effective first-line drug, rifampin. Out of these 600,000 new cases, 490,000 were MDR-TB. Consequently, there is a need for the development of new drugs/products to treat and prevent TB. Sanchez & Kouznetsov [16] state that the discovery and development of new anti-TB drugs are needed for many reasons: (1) to improve the current treatment by shortening its duration and/or providing more widely spaced intermittent treatments, (2) to improve the treatment of MDR-TB and of extensively drug-resistant (XDR-TB) strains, (3) to provide the most effective treatment of latent tuberculosis infection (LTBI) in programs as proposed by the Centers for Disease Control and Prevention (CDC) [17], (4) to reduce the adverse effects, especially hepatotoxicity, which is very important as it leads to forced treatment termination [6,18,19], and (5) finally, because there are only few new drugs on the market since the 1960s [16]. The discovery of new drugs involves essentially the identification of new chemical entities (NCEs) that display the required characteristics of druggability and medicinal chemistry [20]. NCEs can be generated either through chemical synthesis or by isolation from screening natural products. Six classes of sources for NCEs have been reported [20]. Four classes are related to natural products, i.e. from botanical sources, fungi, bacteria, and marine sources. Besides, modern pharmaceutical chemistry has added two categories of man-made substances, i.e. synthetic chemistry and combinatorial chemistry [20].

About 80% of the population in developing countries relies on traditional medicine (TM) for their primary healthcare [21]. TM is mostly used because of its affordability and accessibility [21,22].

Research on the application/utilization of medicinal plants for drug discovery usually starts with an ethnobotanical survey. As such, the selection of a candidate species for investigation can be done because of its long-term use by humans [20]. The idea behind this approach is that the active compounds isolated from such plants are likely to be safer than compounds derived from plant species without a history of human use.

Following the survey, the screening of plant extracts, to confirm a certain activity against pathogens, must use reliable assays. Positive and negative controls must be well defined to avoid false positive or negative results. Screening determines the activity of medicinal plant extracts against microbes of interest, expressed as a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Therefore, the screening of plant extracts is considered an important starting point for antimicrobial drug discovery and development [16]. This preliminary approach using the whole cell of pathogens provides the antimicrobial activity of the crude plant extracts, which contain many different chemical compounds. Bioassay-guided fractionation of the crude extracts may lead to either standardization of extracts or isolation of bioactive druggable compounds for new drugs [20,23,24]. After isolation of the compound from a medicinal plant, the mechanisms by which it inhibits bacterial growth needs to be understood [25]. This can be done by testing the active compounds on known/valid targets. For *M. tuberculosis* potential drug targets such as cell membranes, specific enzymes for transcription and replication, and ATPase were reviewed in Ref. [26] and one can test the isolated compound or the standardized extracts on them. Otherwise, metabolomics can be applied

to assess the global change in terms of metabolism as a response to treatment with that compound or extract. This approach has been used to elucidate the mechanisms of action of bioactive extract or isolated compounds [27–31]. Untargeted metabolomics with liquid chromatography–mass spectrometry [30] allowed exploring the mycobacterial response to cinnamaldehyde with cinnamon essential oil. Predictive metabolite analysis and description of the produced lipids enabled the evaluation of the stress symptoms shown by bacteria. Bacteria exposed to cinnamaldehyde were found to reorganize their outer membrane as a physical barrier against stress factors. They probably reduced the cell wall permeability and the inner membrane fluidity, and possibly redirected the carbon flow to store energy in triacylglycerols. In addition, cinnamaldehyde may also contribute to disturbances in bacterial redox homeostasis and detoxification mechanisms [30]. An approach to predict the *in vivo* mechanisms of action of novel drug leads from NMR metabolomics data is described in Ref. [29]. *M. smegmatis*, a nonpathogenic model organism of *M. tb*, was treated with 12 known drugs and 3 chemical leads identified from a cell-based assay. NMR analysis of drug-induced changes to the *M. smegmatis* metabolome resulted in distinct clustering patterns in orthogonal projections to latent structures discriminant analysis (OPLS-DA) scores plot correlating with *in vivo* drug activity. The clustering of novel chemical leads relative to known drugs provides a means to identify a protein target or to predict *in vivo* activity [29]. The above examples illustrate that metabolomics can be useful in an attempt to study the mechanisms of action and efficacy of novel compounds.

To resorb after oral administration, active compounds must have molecular properties that obey, for instance, the Lipinski Rule of Five [25,32]. To define hits the generation of dose-response curves, specificity regarding structure-activity relationship (SAR), properties concerning absorption, distribution, metabolism and excretion (ADME), as well as physicochemical and pharmacokinetic (PK) measurements, can be tested [23–25]. A hit-to-lead phase needs to be expected prior to lead optimization. The aim of this stage is to try to produce more potent and selective compounds that possess adequate PK properties adequate to examine their efficacy *in vivo* models [24,25]. Practically, the work involves SAR investigations around each isolated compound, with measurements to establish the magnitude of its activity and selectivity [25]. When structural information about the target is known, structure-based drug design techniques, using molecular modeling and methodologies such as X-ray crystallography and nuclear magnetic resonance (NMR), can be applied to develop the SAR faster and in a more focused way.

Toxicity tests must be conducted to confirm the safety of the isolated compound or standardized extracts applying suitable *in vitro* and *in vivo* models [23–25]. However, defining the dose range, minimum concentration and controls to ensure the quality and validity of these tests is challenging. Standardized methods for phytochemical analysis and toxicity tests should be used. Many medicinal plants in the treatment of tuberculosis in Africa were discovered using ethnobotanical and ethnopharmacological approaches [33]. Active ingredients from plant products can also be used as adjuvants to augment the efficacy of existing drugs [33]. Medicinal plants from different species have shown activity against mycobacteria, including *M. tuberculosis*, the causative agent of TB. Despite this knowledge, no anti-TB drug has been recently developed from medicinal plants. In fact, the current strategy to first screen for activity and later isolate the active compound using bioactivity-guided assays is laborious and time-consuming. Besides, this procedure may result in the isolation of already known compounds [34]. A new strategy, determining the metabolite profile or fingerprint of medicinal plants may provide an alternative to the bioactivity-guided fractionation and isolation of active compounds. Metabolite fingerprinting allows the determination of the fingerprints of plant metabolites and eventually the indication of the compounds responsible for the biological activity [35]. Comparing the fingerprints from selected medicinal plant with known bioactivity may indicate the plants with suitable active compounds [36]. This helps identifying common/different

compounds that are responsible for the biological activity. To achieve this, a metabolomics approach that aims at simultaneously measuring as many metabolites as possible in the fingerprint profiles from a representative set of plant samples, can be applied [37]. In this paper, we overview and discuss the antimycobacterial activity, phytochemical analysis, and safety of *Zanthoxylum leprieurii*, *Lantana camara*, and *Cryptolepis Sanguinolenta*, and highlight the importance of metabolomic fingerprinting of medicinal plants linked to activity measurements on microbes. To be able to tackle the burden of TB, new drugs that can treat MDR-TB need to be developed. *Zanthoxylum leprieurii*, *Lantana camara*, and *Cryptolepis Sanguinolenta* were selected because they showed activity on the rifampicin-resistant strain of *M. tb* [38–40] which has been reported a good indicator of MDR *M. tb* [40]. Recently, the application of metabolomics to drug discovery and understanding the mechanisms of action of medicinal plants with anti-tuberculosis activity has been discussed [26]. Metabolic profiling provides the potential to determine the mechanism of action of medicinal plants extracts or isolates [26,29,30], as well as to determine the active compounds from different plants with the same biological activity [29,30,41]. As mentioned above, this review aims at overviewing and discussing the medicinal usage, anti-mycobacterial activity, phytochemistry and safety of *Zanthoxylum leprieurii*, *Lantana camara* and *Cryptolepis Sanguinolenta* as potential candidates for novel multidrug resistant tuberculosis drugs among the in Uganda commonly applied plants. Further, the application of their metabolomic fingerprinting as a tool to determine the compounds potentially responsible for the biological activity is highlighted.

2. *Zanthoxylum leprieurii*

Zanthoxylum leprieurii species (also known as Munyenye in Luganda, Fig. 1) belongs to the *Zanthoxylum* genus (with about 549 species) and the Rutaceae family, which are distributed worldwide [42]. Traditional use of *Zanthoxylum leprieurii* in Africa includes the treatment of HIV/AIDS, malaria, urinary infections, rheumatic pain and as antiseptic [39]. In Uganda, it was reported to be traditionally used to treat tuberculosis and cough-related infections [42–44]. Local communities pound the stem barks and add water, and drink the extract. To the best of our knowledge, only one study has been conducted to determine antimycobacterial activity [39]. Methanolic crude extracts, fractions and active compounds of the stem bark of *Z. leprieurii* Guill. et Perr., collected from Mpigi District in Central Uganda (0° 13' 38.4708" N 32° 19' 29.7264" E), were tested on different strains of *M.tb*. They included a rifampicin-resistant strain (TMC 331/ATCC35838), an isoniazid-resistant strain (TMC 303/ATCC 35822), and a pan-sensitive strain (H37Rv). Table 1 summarizes the results. Only those for total crude methanolic extract and active compounds are presented (see



Fig. 1. *Zanthoxylum leprieurii*. A. Leaves, B. Stem with barks, C. Roots.

Ref. [39] for more detailed information).

The table shows that the MIC values of the active compounds are lower than that of the crude methanol extract. The lower TB inhibition exhibited by the methanolic crude extract compared to the isolate could reflect low amounts of the active molecules. After bioactivity-guided fractionation, the most active molecules were identified. This clearly shows the presence of single active molecules [45]. Three acridone alkaloids were isolated: 2-hydroxy-1,3-dimethoxy-10-methyl-9-acridone (1), 1-hydroxy-3-methoxy-10-methyl-9-acridone (2), and 3-hydroxy-1,5,6-trimethoxy-9-acridone (3). Compound 1 has the lowest MICs, i.e. 1.5 µg/mL, 3.5 µg/mL and 8.3 µg/mL on the pan sensitive, isoniazid-resistant and rifampicin-resistant strains, respectively. The standard anti-mycobacterial drugs showed lower activity against the pan-sensitive strain (MIC = 2 µg/mL for isoniazid and MIC = 4 µg/mL for rifampin) [39]. Note that there were no isoniazid and rifampicin activities toward isoniazid-resistant and rifampicin-resistant strains [39].

The results show that this compound might be developed as an alternative anti-TB drug for multidrug-resistant *M. tuberculosis*. However, the journey to the development of new drugs is multistage, long and costly [20,25]. Therefore, extensive work is needed to meet the requirements of drug discovery and development [24,25,46]. The fact that compound 2 is less active could be explained by different positions of hydroxyl and methoxy functional groups [47]. The literature does not report other active phytochemicals from *Z. leprieurii*.

There is no *in vivo* evidence on the safety of the methanolic crude extract of the roots of *Z. leprieurii*. However, the brine shrimp (*Artemia salina*) lethality bioassay of the chloroform extract of the fruits showed modest cytotoxicity with LD50 at 13.1 µg/mL [44]. Acridone alkaloids from *Z. leprieurii* showed a moderate cytotoxic effect (IC50 of 86 µM) against WRL-68 (liver cancer cell line) [44,48]. Computational approaches, quantitative structure-activity relationships (SAR) and modeling studies have revealed that the acridone alkaloids inhibit the glycosyltransferase and aromatase enzymes of liver cells [48]. Acridone alkaloids have sufficient hydrophilic-lipophilic balance, which allows them to cross the biological membrane and reach the nucleus. They were shown to have nuclease, antiherpes, antimalarial, antileishmanial and anticancer activities [49]. So far, only an *in vitro* model of antimycobacterial activity has been tested. Therefore, more *in vitro* as well as *in vivo* studies are needed to determine the efficacy of *Z. leprieurii* against *M. tuberculosis* because of the plant's potential activity against *M. tuberculosis* strains. These studies should take into consideration *Z. leprieurii* material from different regions, ages and seasons. This is important because the metabolites may vary with soil, age and season. Besides, metabolomics principles could be applied to establish the

Table 1
Minimum inhibitory concentrations (MICs) in µg/mL of total methanol extracts and active molecules isolated from *Zanthoxylum leprieurii* against *M. tuberculosis* strains [39].

	Pan-sensitive strain H37Rv	Rifampicin-resistant strain TMC 331/ ATCC35838	Isoniazid-resistant strain TMC 303/ATCC 35822
Total Methanol crude Extract	47.5	75.3	125.0
Compound 1: 2-hydroxy-1, 3-dimethoxy-10-methyl-9-acridone	1.5	8.3	3.5
Active compound 2: 1-hydroxy-3-methoxy-10-methyl-9-acridone	> 6.25	> 6.25	> 6.25
Active compound 3: 3-hydroxy-1, 5, 6-trimethoxy-9-acridone	5.1	4.5	3.9

metabolite fingerprints of this plant to further study potentially interesting compounds.

3. *Lantana camara*

Lantana camara (*L. camara*) (locally known as Omuhukye) (Fig. 2), a plant that belongs to the Verbenaceae family was also reported to treat tuberculosis [33]. This plant is widely distributed in the East African region. *L. camara* can be found in arid regions and is known to pose a threat to other biodiversities [40]. In addition to the treatment of TB, *L. camara* has many other medicinal applications. *L. camara* was reported to have chemical compounds with antimicrobial, fungicidal, nematocidal and insecticidal activities [40,50,51]. A compound, verbascoside, isolated from *Lantana*, has antimicrobial, immunosuppressive and antitumor activities [40,52]. The antimycobacterial activity and acute toxicity of *L. camara* leaves were investigated in Ref. [40,52]. From anecdotal experience, *L. camara* is allergenic and causes rashes when handling this plant. In the community, the leaves are chewed with salt to treat common cough in humans and cattle. To prove the antimycobacterial activity of *L. camara*, one study was conducted [40]. In this *in vitro* study, *L. camara* leaves, collected in Southwestern Uganda, were extracted with methanol, chloroform, and water, and tested for antimycobacterial activity. Three *M. tb* strains, a wild rifampicin-sensitive 28–25271, a rifampicin-resistant TMC-331, and a pan-sensitive H37Rv strain, were used. The methanolic extract showed the highest activity with MIC values of 20 µg/mL for H37Rv, and 15 µg/mL for the TMC-331 and the wild, (28–25271) strains. Rifampicin showed MIC values of 1.0 µg/mL for the H37Rv and wild strains, but was ineffective against the rifampicin-resistant TMC-331 strain. Rifampicin showed complete growth inhibition for H37Rv and the wild strain at 1.5 µg/mL, but was unable to inhibit TMC-331 even at a concentration of 3.0 µg/mL. A clear contrast was seen with the methanolic extract of *L. camara*, which was active against all *M. tb* strains used [40]. *L. camara*



Fig. 2. *Lantana camara*. A. Flowers, B. Leaves, C. Fruits.

methanolic extract thus contains active ingredients that may be used as anti-TB drugs for MDR *M. tb*. The MIC values of the methanolic extract were higher, probably because of the lower amounts of the active compounds.

An earlier phytochemical analysis reported that *L. camara* produces triterpenoids, such as camaric and rehmamic acids [53]. These compounds are known to have antimycobacterial activity [54]. Other secondary metabolites from *L. camara* were reported [55], but they have not been tested for antimycobacterial activity. Those metabolites include tannins, saponins, flavonoids, cardiac glycosides and alkaloids [56,57]. All research studies were done *in vitro* and confirmed that *L. camara* contains compounds that might be further studied, and eventually used as novel drugs for *M. tb*, including MDR strains. *In vivo* studies are needed to confirm the *in vitro* findings. Further, mechanisms of action are to be identified to clarify the specific targets of *L. camara*.

L. camara is among the most important medicinal plants in the world [56], but is also considered a noxious weed [58–60]. Nevertheless, the acute toxicity profile of the methanolic extract of *L. camara* showed that when pairs of mice (male and female) were given oral doses, the median lethal dose was found to be above 500 mg/kg body weight. The mice experienced sedation for about 6 h at a dose of 500 mg/kg body weight, but there were no anesthetic or analgesic effects, as the sedated animals still responded to a pinch on the tail. An increased breathing rate and restlessness were also observed at doses of 100 mg/kg body weight and above. All animals showed normal activity 24 h after administration [40].

4. *Cryptolepis sanguinolenta*

This plant has been reported to treat tuberculosis in Uganda and other countries [7,22,61,62]. *Cryptolepis sanguinolenta* (*C. sanguinolenta* known as Karondorondo in Luganda, Fig. 3) belongs to the family of the Periplocaceae, synonymous with the family of the Apocynaceae [63]. It is a slender climber up to 25 ft (about 8 m) high with greenish-yellow flowers and yellow roots. It occurs in many countries in sub-Saharan Africa, including Uganda [33,38]. This plant is not only traditionally used to treat human tuberculosis but shows also various other medical applications and pharmaceutical activities, as reviewed in Ref. [63]. They include the use as an antimalarial in West African ethnomedicine [63–66], as anticancer [64], antidiarrheal [67], antifertility [68], antimicrobial [69], antifungal [70,71], anti-diabetic [72], anti-inflammatory and analgesic activity [73], and anti-amoebic medicine [74].

The antimycobacterial activity of this plant on various mycobacterial strains (the pan-sensitive H37Rv, the rifampicin-resistant TMC-331 and a wild strain of *Mycobacterium avium*, isolated from an Ugandan patient) was tested [38]. Roots of *C. sanguinolenta* were harvested from the



Fig. 3. *Cryptolepis sanguinolenta*. A. Leaves, B. Roots (yellow). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Kayunga District in Central Uganda at 1207 m, 01°13'N32°52'E. *C. sanguinolenta* total crude methanolic extract showed the highest activity against H37Rv and TM-331 with complete clearance of quadrants at 50 mg/mL. However, it was not effective against the wild strain *Mycobacterium avium*. The MICs were 1.17 mg/mL for H37Rv and 1.56 mg/mL for TMC-331. The values for isoniazid were 0.25 and 9.38 µg/mL for H37Rv and TMC-331, respectively. The low activity can be explained by the fact that a methanolic total crude extract was used. Pure compounds might have higher activity. *C. sanguinolenta* thus could be a source of compounds that might be developed into drugs to treat MDR TB, given the fact that it has activity on TMC-331, a rifampicin-resistant strain of *M. tb*. However, the preliminary tests applied in early discovery of new drug still need to be performed.

The phytochemical analysis of *C. sanguinolenta* revealed the presence of different secondary metabolites. Alkaloids, tannins, and flavones were found in crude methanolic extract [38]. A study reported that *C. sanguinolenta* root bark contains flavonoids [74]. The difference in reported results of secondary metabolites could be attributed to different methods used for preparing the plant extracts. Cryptolepine alkaloids have been reported as the major alkaloids with antimycobacterial activity in *C. sanguinolenta*. This was confirmed by Refs. [75], using the fast-growing mycobacterial species *M. fortuitum*. In addition to cryptolepine, several active alkaloids, including neocryptolepine, biscryptolepine, cryptolepine and isocryptolepine with antimalarial, antitrypanosomal, antifungal and antimicrobial activities were isolated from the root bark extracts of the plant [65,70,76]. Further investigations are needed to determine the activity of cryptolepine against various virulent strains of *M. tb*.

C. sanguinolenta extracts and cryptolepine alkaloids were analyzed for toxicity. Acute toxicity test on mice gave an LD50 of 759 mg/kg body weight [38]. Using rats, a study by Ansah et al. [77] reported an LD50 of 3000 mg/kg body weight. This difference may result from the fact that rats are probably more tolerant than mice. In addition [38], used methanolic extract, whereas in Refs. [77], the aqueous extract was used. Consequently, *C. sanguinolenta* methanolic extract might contain more pharmacologically active compounds than the aqueous because less polar compounds are not readily soluble in water [38]. *C. sanguinolenta* and its active compound cryptolepine, are thus potential candidates for anti-TB drug development, but need to be extensively studied further.

5. Metabolomic fingerprints of medicinal plants with the same biological activity

Metabolomics aims at qualitatively and quantitatively measuring and analyzing metabolites from biological samples [78–80]. The systematic identification and quantitation of all metabolites in a given organism or biological sample requires a range of analytical tools including molecular detection and bioinformatics to deal with the mountains of data collected [37,81,82]. Scientists use metabolomics to understand systems biology, which is the complete computational analysis and modeling of an organism and its well-being [83–86]. Technically, nuclear magnetic resonance (NMR) and mass spectrometry (MS) are the two main technical approaches used to generate data for metabolomics [37,87–89]. However, hyphenated techniques such as chromatographic techniques coupled to MS or NMR can also be applied [84,88]. Despite the fact that metabolomics technology is highly sophisticated and sensitive, few bottlenecks exist. To date, there is no single technology available, which is able to analyze the entire metabolome of an organism. This is due to the huge diversity of chemical structures and their large differences in abundance [82]. Nevertheless, scientists have developed a number of complementary approaches to be applied for the extraction, detection, quantification, and identification of as many metabolites as possible. Another challenge in metabolomics is to extract the information from the vast amount of data produced by high-throughput analyzers and interpret it in a biological context [82, 90]. Depending on the objective of a researcher, different approaches

can be followed. These include the application of the metabolic profile in a targeted or an untargeted way. The metabolic profile analyzed in a targeted way is a quantitative analysis of a set of metabolites in a selected biochemical pathway or a specific class of compounds [79,82]. The targeted analysis includes the determination of a very limited number of metabolites, for instance, single analytes as precursors or products of biochemical reactions or biomarkers to diagnose diseases. The metabolic fingerprint analyzed in an untargeted way concerns a global screening approach to classify samples based on metabolite patterns or “fingerprints” that change in response to disease, environmental or genetic perturbations with the ultimate goal to identifying discriminating metabolites (biomarkers) [35]. Therefore, the purpose of metabolite fingerprinting is not to identify each observed metabolite but to compare patterns or “fingerprints” of metabolites that change in a given biological system [36]. For plant extracts, the proper choice of a fingerprinting technique depends on the characteristics of the constituents of the plant material [35]. Fingerprinting data combined with chemometric tools have the potential to assess the complex composition of herbal extracts and essential oils. Chemometric tools, including sampling and extraction optimization, design of experiments, exploratory data analysis, data pretreatment, variable selection, regression and pattern-recognition techniques are dedicated to developing and handling plant fingerprints [35,36,41].

Recent advances in untargeted and targeted approaches applied in herbal extracts and essential oils fingerprinting were reviewed in Ref. [41]. The application of fingerprinting may help to rapidly assess the metabolic profiles of medicinal plants with activity on target microorganisms. Metabolites that are potentially active against microorganisms might be identified [24,37,41]. As such, any other plant from the same species with a similar metabolic fingerprint would have a similar activity against the same microorganism. As stated above, the purpose of metabolic fingerprinting is also to compare patterns of metabolites that change in a specific biological system [36]. Thus, comparing medicinal plants with similar biological activity could answer the following questions: i) do plants from different species with the similar biological activity share common compounds? ii) Given the fact that medicinal plants show a different level of bioactivity, does this level of activity reflect the amount or type of active compounds? For instance, the application of fingerprinting to *L. camara*, *Z. lepreurii* and *C. sanguinolenta* may reveal the common or different active compounds that are associated with their antimycobacterial activity. The identification of interesting compounds and discrimination of samples would be done using different chemometrics methods. For instance, principal component analysis (PCA) clusters the samples with a similar metabolic profile [91], while it may show samples outlying to given clusters. Multivariate calibration and classification methods might reveal the metabolites potentially responsible for the observed activity [41,81,92, 93]. Classification, quantification, and identification of relevant biomarkers from the plant metabolome are challenged by the large numbers of plant metabolites with diverse physicochemical properties [94,95]. Therefore, the most commonly used analytical techniques reviewed in Ref. [94,95] cannot cover simultaneously the entire metabolome, as already highlighted before. Each analytical technique has its advantages and bottlenecks in terms of sensitivity, resolution, and reproducibility [94,95]. There is a need to establish a standardized protocol to cover the maximum number of metabolites with reduced time and cost. Some plant metabolites that are sometimes restricted to specific and narrow species within a phylogenetic group [36], are involved in the natural defense against pathogens or in reproducibility of those plants. Therefore, the metabolome of the plant can be influenced by its geographical localization, age and harvesting season [36]. Explaining how different plants from different species families share the same biological activity would be difficult to understand if we could not determine their metabolic fingerprints. Phytochemical analysis of *L. camara* [40,54,56,57,96], *C. sanguinolenta* [63,64,70,71,76,97–99] and *Z. lepreurii* [39,43,44,100,101] revealed the different nature of

their metabolites. Different and specific biological activities have driven such phytochemical analysis. However, to the best of our knowledge, there is no single study available on both the individual and simultaneous metabolic fingerprinting of those plants driven by one biological activity, such as antimycobacterial activity. The bioassay-guided metabolic fingerprinting could help to determine potentially active compounds. Labor, time, and costs associated with classical bioassay-guided fractionation and isolation of active compounds would be reduced. The general workflow and the chemometrics tools discussed in Ref. [41] could be considered for metabolic fingerprinting of *L. camara*, *C. sanguinolenta* and *Z. leprieurii* to determine compounds which are potentially responsible for their antimycobacterial activities.

6. Concluding remarks and perspectives

This paper discusses studies conducted on the antimycobacterial activity, phytochemical analysis and safety of *Zanthoxylum leprieurii*, *Lantana camara* and *Cryptolepis Sanguinolenta*. It shows also how metabolomics tools may be applied to rapidly identify potentially active metabolites in these plants. The literature study showed the potential antimycobacterial activity of crude methanolic extracts of these plants and their active compounds on different strains of *M.tb*, including MDR strains. Thus, they can result in possible future antimycobacterial leads if other tests for early drug discovery as discussed in the text, are met. The three plants are promising sources of potential anti-TB drugs that might eventually eliminate MDR *M.tb* strains. However, extensive work is still needed. The phytochemical analysis showed the presence of different secondary metabolites and active compounds, which is a good start in the search for new druggable compounds. The toxicological data are not conclusive because only *in vitro* toxicity was determined. Further investigations on long-term toxicity are also needed. Compound optimization will be very important as if the compounds found might not have good structural form for making a drug, optimization will help with relevant modifications that will still maintain or improve bioactivity on *M. tuberculosis* strains. In addition, *in vivo* tests of the antimycobacterial activity in the animal model, the safety, biochemical and bioavailability properties, and the elucidation of the mechanisms of action of these compounds are required to validate the *in vitro* studies. Other research studies that include tests for the druggability of extracts and isolated compounds, clinical trials, determination of pharmacodynamics and pharmacokinetics of active compounds would follow as required.

Funding

This study is part of the PhD program that is funded by World Bank and VUB.

Ethical approval

Not required.

Declaration of competing interest

None.

Acknowledgements

The authors express their gratitude to the Vrije Universiteit Brussel (VUB) and World Bank for their funding through Global Minds Scholarship and the PHARMBIOTRAC-Africa Center of Excellence (ACE II), Mbarara University of Science and Technology, respectively.

References

- [1] World Health Organization. Global tuberculosis report 2019. Geneva. 2019. <https://apps.who.int/iris/handle/10665/329368>.

- [2] World Health Organization. Global tuberculosis report 2017. Geneva. 2017. <https://apps.who.int/iris/handle/10665/259366>.
- [3] Nath Jnawali Hum, Ryoo Sungweon. First and second line drugs and drug resistance. *Tuberc. Issues Diagnosis Manag.*; 2013. p. 163–80. <https://doi.org/10.5772/51895>.
- [4] World Health Organization. Global tuberculosis report 2014. Geneva. <https://apps.who.int/iris/handle/10665/137094>; 2014.
- [5] Vilarica AS, Diogo N, André M, Pina J. Adverse reactions to antituberculosis drugs in in-hospital patients: severity and risk factors. *Rev Port Pneumol* 2010;16: 431–51. [https://doi.org/10.1016/S0873-2159\(15\)30040-4](https://doi.org/10.1016/S0873-2159(15)30040-4).
- [6] Forget EJ, Menzies D. Adverse reactions to first-time antituberculosis drugs. *Expet Opin Drug Saf* 2006;5:231–49. <https://doi.org/10.1517/14740338.5.2.231>.
- [7] Arya V. A review on anti-tubercular plants. *Int J PharmTech Res* 2011;3:872–80. <https://doi.org/10.1177/0300985811429313>.
- [8] El-Din MAT, Halim HAA-E, El-Tantawy AM. Adverse reactions among patients being treated for multidrug-resistant tuberculosis in Egypt from July 2006 to January 2009. *Egypt J Chest Dis Tuberc* 2015;64:657–64. <https://doi.org/10.1016/j.ejcdt.2015.05.011>.
- [9] Pooran A, Pieterse E, Davids M, Theron GDK. What is the cost of diagnosis and management of drug resistant tuberculosis in South Africa? *PLoS One* 2013;8: e54587. <https://doi.org/10.1371/journal.pone.0054587> LK.
- [10] Ordas A, Raterink RJ, Cunningham F, Jansen HJ, Wiweger MI, Jong-Raadsen S, et al. Testing tuberculosis drug efficacy in a zebrafish high-throughput translational medicine screen. *Antimicrob Agents Chemother* 2015;59:753–62. <https://doi.org/10.1128/AAC.03588-14>.
- [11] Marks SM, Flood J, Seaworth B, Hirsch-Moverman Y, Armstrong L, Mase S, Sheeran K. Treatment practices, outcomes, and costs of multidrug-resistant and extensively drug-resistant tuberculosis, United States, 2005–2007. *Emerg Infect Dis* 2014;20:812–21. <https://doi.org/10.3201/eid2005.131037>.
- [12] Hoppe LE, Kettle R, Eisenhut M, Abubakar I. Tuberculosis - diagnosis, management, prevention, and control: summary of updated NICE guidance. *BMJ* 2016;352. <https://doi.org/10.1136/bmj.h6747>. h6747. 13.
- [13] Mitnick CD, White RA, Lu C, Rodriguez CA, Bayona J, Becerra MC, et al. Multidrug-resistant tuberculosis treatment failure detection depends on monitoring interval and microbiological method. *Eur Respir J* 2016;48:1160–70. <https://doi.org/10.1183/13993003.00462-2016>.
- [14] Awasthi D, Freundlich JS. Antimycobacterial metabolism: illuminating *Mycobacterium tuberculosis* biology and drug discovery. *Trends Microbiol* 2017; 25:756–67. <https://doi.org/10.1016/j.tim.2017.05.007>.
- [15] Raymond B. Five rules for resistance management in the antibiotic apocalypse, a road map for integrated microbial management. *Evol Appl* 2019;12:1079–91. <https://doi.org/10.1111/eva.12808>.
- [16] Sanchez JGB, Kouznetsov VV. Antimycobacterial susceptibility testing methods for natural products research. *Braz J Microbiol* 2010;41:270–7. <https://doi.org/10.1590/S1517-83822010000200001>.
- [17] Sterling TR, Njie G, Zenner D, Cohn DL, Reves R, Ahmed A, et al. Guidelines for the treatment of latent tuberculosis infection: recommendations from the national tuberculosis controllers association and CDC. *Am J Transplant* 2020;20: 1196–206. <https://doi.org/10.1111/ajtm.12020>.
- [18] An HR, Wu XQ. Antituberculosis drugs and hepatotoxicity. *Chin J Antibiot* 2010; 10. 4.
- [19] Park JS, Cho YJ, Yoon H II, Song J, Lee CT, Lee JH. Drug-induced hepatotoxicity of anti-tuberculosis drugs and their serum levels. *J Kor Med Sci* 2015;30:167–72. <https://doi.org/10.3346/jkms.2015.30.2.167>.
- [20] Katiyar C, Gupta A, Kanjilal S, Katiyar S. Drug discovery from plant sources: an integrated approach. *Ayu* 2012;33:10–9. <https://doi.org/10.4103/0974-8520.100295>.
- [21] Kasilo O, Trapsida J. An overview of the traditional medicine situation in the African Region. *African Heal Monit* 2010;14:7–15.
- [22] Gupta R, Thakur B, Singh P, Singh HB, Sharma VD, Katoch VM, et al. Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates. *Indian J Med Res* 2010;131:809–13.
- [23] Sittampalam GS, Coussens NP, Brimacombe K, Grossman A, Arkin M, Auld D, et al. Early drug discovery and development guidelines: for academic researchers, collaborators, and start-up companies. *Assay Guid Man*; 2018. <https://www.ncbi.nlm.nih.gov/books/%0ANBK53196>. [Accessed 10 July 2020].
- [24] Nguta JM, Appiah-Opong R, Nyarko AK, Yeboah-Manu D, Addo PGA. Current perspectives in drug discovery against tuberculosis from natural products. *Int J Mycobacteriology* 2015;4:165–83. <https://doi.org/10.1016/j.ijmyco.2015.05.004>.
- [25] Hughes JP, Rees SS, Kalindjian SB, Philpott KL. Principles of early drug discovery. *Br J Pharmacol* 2011;162:1239–49. <https://doi.org/10.1111/j.1476-5381.2010.01127.x>.
- [26] Tuyiringire N, Tusubira D, Munyampundu J-P, Tolo CU, Muvunyi CM, Ogwang PE. Application of metabolomics to drug discovery and understanding the mechanisms of action of medicinal plants with anti-tuberculosis activity. *Clin Transl Med* 2018;7. <https://doi.org/10.1186/s40169-018-0208-3>. 29.
- [27] Jansen RS, Rhee KY. Emerging approaches to tuberculosis drug development: at home in the metabolome. *Trends Pharmacol Sci* 2017;38:393–405. <https://doi.org/10.1016/j.tips.2017.01.005>.
- [28] Prosser GA, De Carvalho LPS. Metabolomics reveal D-alanine: D-alanine ligase as the target of D-cycloserine in *Mycobacterium tuberculosis*. *ACS Med Chem Lett* 2013;4:1233–7. <https://doi.org/10.1021/ml400349n>.

- [29] Halouska S, Fenton RJ, Barletta RG, Powers R. Predicting the in vivo mechanism of action for drug leads using NMR metabolomics. *ACS Chem Biol* 2012;7: 166–71. <https://doi.org/10.1021/cb200348m>.
- [30] Sieniawska E, Sawicki R, Golus J, Georgiev ML. Untargeted metabolomic exploration of the *Mycobacterium tuberculosis* stress response to cinnamon essential oil. *Biomolecules* 2020;10. <https://doi.org/10.3390/biom10030357>. 357.
- [31] Prosser GA, Rodenburg A, Khoury H, De Chiara C, Howell S, Snijders AP, et al. Glutamate racemase is the primary target of β -chloro-D-alanine in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2016;60:6091–9. <https://doi.org/10.1128/AAC.01249-16>.
- [32] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 2012;64:4–17. <https://doi.org/10.1016/j.addr.2012.09.019>.
- [33] Sharifi-Rad J, Salehi B, Stojanović-Radić ZZ, Fokou PVT, Sharifi-Rad M, Mahady GB, et al. Medicinal plants used in the treatment of tuberculosis - ethnobotanical and ethnopharmacological approaches. *Biotechnol Adv* 2017; S0734–9750. <https://doi.org/10.1016/j.biotechadv.2017.07.001>. 30077–0.
- [34] Lu L, Wang J, Xu Y, Wang K, Hu Y, Tian R, et al. A high-resolution LC-MS-based secondary metabolite fingerprint database of marine bacteria. *Sci Rep* 2014;4: 1–7. <https://doi.org/10.1038/srep06537>.
- [35] Mattoli L, Cangi F, Maidecchi A, Ghiara C, Ragazzi E, Tubaro M, et al. Metabolomic fingerprinting of plant extracts. *J Mass Spectrom* 2006;41:1534–45. <https://doi.org/10.1002/jms.1099>.
- [36] Wolfender J, Rudaz S, Choi YH, Kim HK. Plant metabolomics: from holistic data to relevant biomarkers. *Curr Med Chem* 2013;20:1056–90. <https://doi.org/10.2174/0929867311320080009>.
- [37] Alonso A, Marsal S, Julià A. Analytical methods in untargeted metabolomics: state of the art in 2015. *Front Bioeng Biotechnol* 2015;3:1–20. <https://doi.org/10.3389/fbioe.2015.00023>.
- [38] Kirimuhuzya C, Bunalema L, Waako P, Tabuti JRS, Orodho J, Magadula JJ, et al. Efficacy of *Cryptolepis sanguinolenta* root extract on slow-growing rifampicin resistant *Mycobacterium tuberculosis*. *J Med Plants Res* 2012;6:1140–6. <https://doi.org/10.5897/JMPR10.856>.
- [39] Bunalema L, Fotso GW, Waako P, Tabuti J, Yeboah SO. Potential of *Zanthoxylum leprieurii* as a source of active compounds against drug resistant *Mycobacterium tuberculosis*. *BMC Compl Alternative Med* 2017;17:4–9. <https://doi.org/10.1186/s12906-017-1602-x>.
- [40] Kirimuhuzya C, Waako P, Joloba M, Odyek O. The anti-mycobacterial activity of *Lantana camara* a plant traditionally used to treat symptoms of tuberculosis in South-western Uganda. *Afr Health Sci* 2009;9:40–5. <https://doi.org/10.4314/ahs.v9i1.7101>.
- [41] Kharbach M, Marmouzi I, El M, Bouklouze A, Vander Y. Recent advances in untargeted and targeted approaches applied in herbal-extracts and essential-oils fingerprinting - a review. *J Pharmaceut Biomed Anal* 2020;177. <https://doi.org/10.1016/j.jpba.2019.112849>. 112849.
- [42] Lamorde M, Tabuti JRS, Obua C, Kukunda-Byobona C, Lanyero H, Byakika-Kibwika P, et al. Medicinal plants used by traditional medicine practitioners for the treatment of HIV/AIDS and related conditions in Uganda. *J Ethnopharmacol* 2010;130:43–53. <https://doi.org/10.1016/j.jep.2010.04.004>.
- [43] Misra LN, Wouatsa NAV, Kumar S, Venkatesh Kumar R, Tchoumboungang F. Antibacterial, cytotoxic activities and chemical composition of fruits of two Cameroonian *Zanthoxylum* species. *J Ethnopharmacol* 2013;148:74–80. <https://doi.org/10.1016/j.jep.2013.03.069>.
- [44] Ngoumfo RM, Jouda J-BB, Mouafo FT, Komguem J, Mbazoa CD, Shiao TC, et al. *In vitro* cytotoxic activity of isolated acridones alkaloids from *Zanthoxylum leprieurii* Guill. et Perr. *Bioorg Med Chem* 2010;18:3601–5. <https://doi.org/10.1016/j.bmc.2010.03.040>.
- [45] Akintola AO, Kehinde AO, Adebisi OE, Ademowo OG. Anti-tuberculosis activities of the crude methanolic extract and purified fractions of the bulb of *Crinum jagus*. *Niger J Physiol Sci* 2013;28:135–40.
- [46] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 2001;46:3–26. [https://doi.org/10.1016/s0169-409x\(00\)0129-0](https://doi.org/10.1016/s0169-409x(00)0129-0).
- [47] Yadav AK, Thakur J, Prakash O, Khan F, Saikia D, Gupta MM. Screening of flavonoids for antitubercular activity and their structure–activity relationships. *Med Chem Res* 2013;22:2706–16. <https://doi.org/10.1007/s00044-012-0268-7>.
- [48] Wouatsa VNA, Misra L, Kumar S, Prakash O, Khan F, Tchoumboungang F, et al. Aromatase and glycosyl transferase inhibiting acridone alkaloids from fruits of Cameroonian *Zanthoxylum* species. *Chem Cent J* 2013;7. <https://doi.org/10.1186/1752-153X-7-125>. 125.
- [49] Michael JP. Acridone alkaloids. In: J Knölker H, editor. *The alkaloids chemistry and biology*, vol. 78; 2017. p. 1–108. <https://doi.org/10.1016/bs.alkal.2017.06.001>.
- [50] Ghisalberti EL. *Lantana camara* L. (Verbenaceae). *Fitoterapia* 2000;71:467–86. [https://doi.org/10.1016/S0367-326X\(00\)00202-1](https://doi.org/10.1016/S0367-326X(00)00202-1).
- [51] Kalita S, Kumar G, Karthik L, Rao KVB. A review on medicinal properties of *Lantana camara* linn. *Res J Pharm Technol* 2012;5:711–5. <https://doi.org/10.1155/2016/4104595>.
- [52] Pour BM, Sasidharan S. *In vivo* toxicity study of *Lantana camara*. *Asian Pac J Trop Biomed* 2011;1:230–2. [https://doi.org/10.1016/j.s2221-1691\(11\)60033-6](https://doi.org/10.1016/j.s2221-1691(11)60033-6).
- [53] Jimenez-Arellanes A, Meckes M, Ramirez R, Torres J, Luna-Herrera J. Activity against multidrug-resistant *Mycobacterium tuberculosis* in Mexican plants used to treat respiratory diseases. *Phyther Res* 2003;17:903–8. <https://doi.org/10.1002/ptr.1377>.
- [54] Wächter GA, Valcic S, Franzblau SG, Suarez E, Timmermann BN. Antitubercular activity of triterpenoids from *Lippia turbinata*. *J Nat Prod* 2001;64:37–41. <https://doi.org/10.1021/np000267b>.
- [55] Murrugesan S, Senthilkumar N, Suresh Babu D, Rajasugunasekar D. Chemical constituents and toxicity assessment of the leaf oil of *Lantana camara* Linn from Tamilnadu regions. *Asian J Plant Sci Res* 2016;6:32–42.
- [56] Sharma OP, Singh A, Sharma S. Levels of lantadenes, bioactive pentacyclic triterpenoids, in young and mature leaves of *Lantana camara* var. aculeata. *Fitoterapia* 2000;71:487–91. [https://doi.org/10.1016/S0367-326X\(00\)00156-8](https://doi.org/10.1016/S0367-326X(00)00156-8).
- [57] Mamta S, Jyoti S. Phytochemical screening acorus *Calamus* and *Lantana camara*. *Pharmacy* 2012;3:324–6.
- [58] Mello FB, Jacobus D, Carvalho K, Mello JRB. Effects of *Lantana camara* (Verbenaceae) on general reproductive performance and teratology in rats. *Toxicol* 2005;45:459–66. <https://doi.org/10.1016/j.toxicol.2004.12.004>.
- [59] De Mello FB, Jacobus D, Cristina K, De Carvalho S, De Mello JRB. Effects of *Lantana camara* (verbenaceae) on rat fertility. *Vet Hum Toxicol* 2003;45:20–3.
- [60] Maurício Pereira J, Barreto RW, Ellison CA, Maffia LA. *Corynespora cassiicola* f. sp. *lantanae*: a potential biocontrol agent from Brazil for *Lantana camara*. *Biol Contr* 2003;26:21–31. [https://doi.org/10.1016/S1049-9644\(02\)00112-3](https://doi.org/10.1016/S1049-9644(02)00112-3).
- [61] Orodho JA, Okemo Tabuti, Otieno, Magadula, Kirimuhuzya. Indigenous knowledge of communities around Lake Victoria Basin regarding treatment and management of tuberculosis using medicinal plants. *Int J Med Med Sci* 2014;6: 16–23. <https://doi.org/10.5897/IJMMS09.374>.
- [62] Semenyi SS, Maroyi A. Medicinal plants used for the treatment of tuberculosis by Bapedi traditional healers in three districts of the Limpopo Province, South Africa. *Afr J Tradit, Complementary Altern Med* 2013;10:316–23.
- [63] Osafo N, Mensah KB, Yeboah OK. Phytochemical and pharmacological review of *Cryptolepis sanguinolenta* (lindl.) schlechter. *Adv Pharmacol Sci* 2017;2017. <https://doi.org/10.1155/2017/3026370>. 3026370.
- [64] Anshah C, Mensah KB. A review of the anticancer potential of the antimalarial herbal *Cryptolepis sanguinolenta* and its major alkaloid cryptolepine. *Ghana Med J* 2013;47:137–47.
- [65] Tona L, Ngimbi NP, Tsakala M, Mesia K, Cimanga K, Apers S, et al. Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. *J Ethnopharmacol* 1999;68:193–203. [https://doi.org/10.1016/S0378-8741\(99\)00090-2](https://doi.org/10.1016/S0378-8741(99)00090-2).
- [66] Anshah C, Gooderham NJ. The popular herbal antimalarial, extract of *Cryptolepis sanguinolenta*, is potentially cytotoxic. *Toxicol Sci* 2002;70:245–51. <https://doi.org/10.1093/toxsci/70.2.245>.
- [67] Paulo A, Pimentel M, Viegas S, Pires I, Duarte A, Cabrita J, et al. *Cryptolepis sanguinolenta* activity against diarrhoeal bacteria. *J Ethnopharmacol* 1994;44: 73–7. [https://doi.org/10.1016/0378-8741\(94\)90071-X](https://doi.org/10.1016/0378-8741(94)90071-X).
- [68] Akhigbe R, Ajayi A. Antifertility activity of *Cryptolepis sanguinolenta* leaf ethanolic extract in male rats. *J Hum Reprod Sci* 2012;5:43–7. <https://doi.org/10.4103/0974-1208.97799>.
- [69] Agboke AA, Attama AA, Momoh MA. Evaluation of the antimicrobial activities of crude extract of *Cryptolepis sanguinolenta* and *Cratogeomys adansonii* leaves and their interactions. *J Appl Pharmaceut Sci* 2011;1:85–9.
- [70] Cimanga K, De Bruyne T, Pieters L, Totte J, Tona L, Kambu K, et al. Antibacterial and antifungal activities of neocryptolepine, biscryptolepine and cryptolepine, alkaloids isolated from *Cryptolepis sanguinolenta*. *Phytomedicine* 1998;5:209–14. [https://doi.org/10.1016/S0944-7113\(98\)80030-5](https://doi.org/10.1016/S0944-7113(98)80030-5).
- [71] Sawyer IK, Berry MI, Brown MW, Ford JL. The effect of cryptolepine on the morphology and survival of *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae*. *J Appl Bacteriol* 1995;79:314–21. <https://doi.org/10.1111/j.1365-2672.1995.tb03143.x>.
- [72] Akhigbe R, Okeleji L, Olaleye S, Ajayi A, Adewumi O, Mujaidu K. Effect of ethanolic extract of *Cryptolepis sanguinolenta* stem on *in vivo* and *in vitro* glucose absorption and transport: mechanism of its antidiabetic activity. *Indian J Endocrinol Metab* 2012;16:S91–6. <https://doi.org/10.4103/2230-8210.94265>.
- [73] Olajide OA, Bhatia HS, De Oliveira ACP, Wright CW, Fiebich BL. Anti-neuroinflammatory properties of synthetic cryptolepine in human neuroblastoma cells: possible involvement of NF- κ B and p38 MAPK inhibition. *Eur J Med Chem* 2013;63:333–9. <https://doi.org/10.1016/j.ejmech.2013.02.004>.
- [74] Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ. Antiamoebic and phytochemical screening of some Congolese medicinal plants. *J Ethnopharmacol* 1998;61:57–65. [https://doi.org/10.1016/S0378-8741\(98\)00015-4](https://doi.org/10.1016/S0378-8741(98)00015-4).
- [75] Gibbons S, Fallah F, Wright CW. Cryptolepine hydrochloride: a potent antimycobacterial alkaloid derived from *Cryptolepis sanguinolenta*. *Phyther Res* 2003;17:434–6. <https://doi.org/10.1002/ptr.1284>.
- [76] Cimanga K, De Bruyne T, Pieters L, Claeys M, Vlietinck A. New alkaloids from *Cryptolepis sanguinolenta*. *Tetrahedron Lett* 1996;37:1703–6. [https://doi.org/10.1016/0040-4039\(96\)00112-8](https://doi.org/10.1016/0040-4039(96)00112-8).
- [77] Anshah C, Otsyina HR, Duwiejua M, Woode E, Aboagye FA, Aning KG. Toxicological assessment of *Cryptolepis sanguinolenta* for possible use in veterinary medicine. *J Vet Med Anim Heal* 2009;1:11–6.
- [78] Robertson DG, Reilly MD. The current status of metabolomics in drug discovery and development. *Drug Dev Res* 2012;73:535–46. <https://doi.org/10.1002/ddr.21047>.
- [79] Idle JR, Gonzalez FJ. Metabolomics. *Cell Metab* 2007;6:348–51. <https://doi.org/10.1016/j.cmet.2007.10.005>.
- [80] Powers R. NMR metabolomics and drug discovery. *Magn Reson Chem* 2009;47: S2–11. <https://doi.org/10.1002/mrc.2461>.

- [81] Worley B, Powers R. Multivariate analysis in metabolomics. *Curr Metabolomics* 2012;1:92–107. <https://doi.org/10.2174/2213235X11301010092>.
- [82] Kasture VS, Musmade DS, Vakte MB, Sonawane SB, Patil PP. Metabolomics: current technologies and future trends. *Int J Res Dev Pharm Life Sci* 2012;2: 206–17.
- [83] Wishart D. Metabolomics in humans and other mammals. In: Villa-Boas SG, Roessner U, Hansen M, Smedsgaard J, Nielsen J, editors. *Metabolome analysis: an introduction*. Hoboken: John Wiley & Sons Inc.; 2007. p. 253–88. <https://doi.org/10.1002/9780470105511.ch10>.
- [84] Gomase VS, Changbhale SS, Patil SA, Kale KV. Metabolomics. *Curr Drug Metabol* 2008;9:89–98. <https://doi.org/10.2174/138920008783331149>.
- [85] Blow N. Metabolomics: biochemistry's new look. *Nature* 2008;455:697–700. <https://doi.org/10.1038/455697a>.
- [86] Zhang A, Sun H, Xu H, Qiu S, Wang X. Cell metabolomics. *OMICS A J Integr Biol* 2013;17:495–501. <https://doi.org/10.1089/omi.2012.0090>.
- [87] Liesenfeld DB, Habermann N, Owen RW, Scalbert A, Ulrich CM. Review of mass spectrometry-based metabolomics in cancer research. *Cancer Epidemiol Biomarkers Prev* 2013;22:2182–201. <https://doi.org/10.1158/1055-9965.EPI-13-0584>.
- [88] Shulaev V. Metabolomics technology and bioinformatics. *Briefings Bioinform* 2006;7: 128–39. <https://doi.org/10.1093/bib/bbl012>.
- [89] Lei Z, Huhman DV, Sumner LW. Mass spectrometry strategies in metabolomics. *J Biol Chem* 2011;286:25435–42. <https://doi.org/10.1074/jbc.R111.238691>.
- [90] Syggelou A, Iacovidou N, Atzori L, Xanthos T, Fanos V. Metabolomics in the developing human being. *Pediatr Clin* 2012;59:1039–58. <https://doi.org/10.1016/j.pcl.2012.07.002>.
- [91] Zhou B, Xiao JF, Tuli L, Ransom HW. LC-MS-based metabolomics. *Mol Biosyst* 2012;8:470–81. <https://doi.org/10.1039/c1mb05350g>.
- [92] Agin A, Heintz D, Ruhland E, Chao de la Barca JM, Zumsteg J, Moal V, et al. Metabolomics - an overview. From basic principles to potential biomarkers (part 1). *Med Nucl* 2016;40:4–10. <https://doi.org/10.1016/j.mednuc.2015.12.006>.
- [93] Putri SP, Nakayama Y, Matsuda F, Uchikata T, Kobayashi S, Matsubara A, et al. Current metabolomics: practical applications. *J Biosci Bioeng* 2013;115:579–89. <https://doi.org/10.1016/j.jbiosc.2012.12.007>.
- [94] Kosmides AK, Kamisoglu K, Calvano SE, Corbett SA, Androulakis IP. Metabolomic fingerprinting: challenges and opportunities. *Crit Rev Biomed Eng* 2013;41: 205–21. <https://doi.org/10.1615/CritRevBiomedEng.2013007736>.
- [95] Segers K, Declercq S, Mangelings D, Vander Heyden Y, Van Eeckhaut A. Analytical techniques for metabolomic studies: a review. *Bioanalysis* 2019;11: 2297–318. <https://doi.org/10.4155/bio-2019-0014>.
- [96] Nischal P, Sharma AD. Chemical fingerprint based involvement of plant metabolites and osmoregulatory solutes in providing abiotic stress tolerance to invasive plant *Lantana camara*. *J Stress Physiol Biochem* 2019;15:93–102.
- [97] Bracca ABJ, Heredia DA, Larghi EL, Kaufman TS. Neocryptolepine (cryptotackeine), a unique bioactive natural product: isolation, synthesis, and profile of its biological activity. *Eur J Org Chem* 2014;2014:7979–8003. <https://doi.org/10.1002/ejoc.201402910>.
- [98] Pousset JL, Martin MT, Jossang A, Bodo B. Isocryptolepine from *Cryptolepis sanguinolenta*. *Phytochemistry* 1995;39:735–6. [https://doi.org/10.1016/0031-9422\(94\)00925-J](https://doi.org/10.1016/0031-9422(94)00925-J).
- [99] Barku VY, Dzotsi EY. Isolation and pharmacological activities of alkaloids from *Cryptolepis sanguinolenta* (Lindl.) Schl. *Int Res J Biochem Bioinforma* 2012;2: 58–61.
- [100] Lawal TO, Adeniyi B, Wan AB, Franzblau SG, Mahady GB. *In-vitro* susceptibility of *Mycobacterium tuberculosis* to extracts of *Uvaria afzelli* Scott Elliot and *Tetracera alnifolia* Willd. *Afr J Biomed Res* 2011;14:17–21.
- [101] Ladino OJP, Suárez LEC. Chemical constituents of the wood from *Zanthoxylum quinduense* Tul. (Rutaceae). *Quim Nova* 2010;33:1019–21. <https://doi.org/10.1590/S0100-40422010000500002>.