



Original Research Article

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***In vitro Antimycobacterial Activity of Selected Medicinal Plants
against *Mycobacterium tuberculosis****

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The objective of this study was to evaluate the antimycobacterial activity of *Carissa edulis*, *Sarcocapnos latifolius*, and *Vitex doniana*, three Togolese medicinal plants, used locally for the management of tuberculosis (TB) against *Mycobacterium tuberculosis*. Hydroethanolic extracts of the roots of *C. edulis* and *S. latifolius*, and leaves of *V. doniana* were obtained by maceration. The *in vitro* antimycobacterial activity was evaluated using Microplate Alamar Blue Assay (MABA). The phytochemical screening of the extracts was performed on the basis of tests for coloring characteristics to identify the major chemical groups. The hydroethanolic extracts of the leaves of *V. doniana* and roots of *C. edulis* and *S. latifolius* had antimycobacterial activity against *M. tuberculosis* with minimum inhibitory concentration (MIC) ranging from 312.5-1,250 µg/mL. The best MIC was obtained for the leaves of *V. doniana*. The extracts of the three plants contain alkaloids, flavonoids, tannins, saponins, and cardiac glycosides except the saponins which are absent in the root of *C. edulis*. The present study supports the local use of these plants in the management of TB. However, further investigations are needed on isolating and identifying chemical constituents responsible for the observed activity, and also on the toxicity of these plants.

Introduction

Tuberculosis (TB) is a communicable disease that is a major cause of ill health, one of the

top 10 causes of death worldwide and the leading cause of death from a single infectious agent. TB is caused by the bacillus *Mycobacterium tuberculosis*, which is spread

when people who are sick with TB expel bacteria into the air; for example, by coughing. The disease typically affects the lungs (pulmonary TB) but can also affect other sites (extrapulmonary TB). About a quarter of the world's population is infected with *M. tuberculosis* (WHO, 2020; Brudey *et al.*, 2006). Globally, in 2019, an estimated 10.0 million people fell ill with TB, a number that has been declining very slowly in recent years. There were an estimated 1.2 million TB deaths among HIV-negative people in 2019, and an additional 208 000 deaths among HIV-positive people (WHO, 2020).

Moreover, drug-resistant TB continues to be a public health threat. Worldwide in 2019, close to half a million people developed rifampicin-resistant TB, of which 78% had multidrug-resistant TB. The increase in the incidence of clinical tuberculosis is associated with increasing reports of new cases of multidrug-resistant (MDR-TB) and extensively multi drug-resistant (XDR-TB) strains (Brudey *et al.*, 2006). Management of TB/MDR-TB patient requires intense multi-chemotherapy for at least six months to two years. It is very hurtful to a patient's health due to high levels of drug toxicity and its adverse effects (Rivoire *et al.*, 2007; Aleme and Gebeyehu, 2010; Hannan *et al.*, 2011). Faced with this situation, the development of new drugs is critical for the future control of tuberculosis. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Hamisi *et al.*, 2009). It is with this in mind that work has focused on plants used in the treatment of several diseases including tuberculosis (Madikizela *et al.*, 2017). Several plants have been identified in the treatment of tuberculosis and other diseases caused by mycobacteria (Madikizela *et al.*, 2017; Makagni *et al.*, 2020). Moreover, higher plant extracts have been considered as

promising sources of novel anti-TB leads (Mitscher and Baker, 1998; Kahaliw *et al.*, 2017). This has prompted us to investigate *Carissa edulis* Vahl (Apocynaceae), *Sarcocephalus latifoliu* (Sm.) E.A. Bruce (Rubiaceae) and *Vitex doniana* Sweet (Lamiaceae), three medicinal plants used in Togo in the management of TB, for possible anti-TB activities. So, the objective of the present study is to evaluate the *in vitro* antimycobacterial activity of hydroalcoholic extracts of these three plants on *Mycobacterium tuberculosis* and then to carry out the phytochemical screening of the tested extracts.

Materials and Methods

Collection of plants materials

The roots of *Carissa edulis* and *Sarcocephalus latifoliu*, and leaves of *Vitex doniana*, were collected from Pagala Gare (Blitta district of Togo) in March 2019. The plants materials were botanically authenticated at the Herbarium of Botanical Department, Faculty of Sciences, University of Lomé.

Preparation of the extract

The extraction was performed according to Hoekou *et al.*, (2017). The samples of each plant were washed thoroughly under running tap water, and dried in air at room temperature for two weeks. After wards, the plant materials were powdered using a grinding machine. The ethanolic extraction was performed by maceration of 100 g powder in 1,000 mL of 70% (v/v) ethanol, under continuous stirring for 48 hours at room temperature. The extract was filtered through Whatman No.1 filter paper and evaporated at 45°C using a rotary evaporator to dryness under reduced pressure. The extracts were preserved at 4°C in refrigerator till used.

***In vitro* antimycobacterial assay**

Bacterial strains

The microorganisms used for the test were clinical strains of *Mycobacterium tuberculosis* isolated from the National Tuberculosis Reference Laboratory of the University Hospital Centre Sylvanus Olympio (CHU-SO) of Lomé.

Microplate alamar blue assay (MABA)

Mycobacterial strains were freshly subcultured on Lowenstein-Jensen (LJ) medium. The inoculum was prepared from a BD BACTEC Mycobacterial Growth Indicator Tube (MGIT) 7 mL positive tube within 5 days. Mix the contents of the tube well by vortexing it, then dilute it to 1/5 by adding 1 mL of culture to 4 mL of sterile sodium chloride (0.9%) and mix well (solution A). Add 0.1 mL of this solution A to 9.9 mL of MGIT supplemented with 0.8 mL of sterile sodium chloride (solution B). Close tightly and mix 2-3 times by turning (BD BBL MGIT, 2016). The microplateal amarblue assay was carried out as described by Palomino *et al.*, (2002). Briefly, 100 µL of MGIT broth was dispensed in each well of a sterile flat-bottom 96-well plate, and serial two fold dilutions of the crude extracts, and isoniazid (INH) an drifampicin (RIF), as positive control drugs, were prepared directly in the plate.

One hundred microliters of inoculum was added to each well. A growth control treated with the vehicle (sterile distilled water) and a sterile control were also included for each mycobacterial strain. Sterile water was added to all perimeter wells to avoid evaporation during the incubation. The plates were covered, sealed with Parafilm, and incubated at 37 °C under a normal atmosphere. After 7 days of incubation, 32.5µL of a mixture of MGIT (1.9225 mL), distilled water (3.0275

mL) and Resazurin (0.05 mL) or alamar blue solution was added to each well, and the plate was reincubated overnight. A change in color from blue to pink indicated the growth of bacteria, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration of the crude extract or positive control drug that prevented this change in color. The crude extract and drug concentration ranges used were as follows: for crude extracts, 5,000 to 9.75 µg/mL, INH, 64 to 0.12 µg/mL and RIF, 83 to 0.16 µg/mL. All the experiments were carried out in triplicate.

Phytochemical analysis

Phytochemical screening was performed on the basis of tests for coloring characteristics to highlight the major chemical groups. It focused on hydroethanolic extracts of the studied plants. The chemical groups were identified by reference to the methods described by Harbone(1976).

Alkaloids

The techniques of Dragendorff and Mayer were used. Two drops of Dragendorff's reagent were added to 2 mL of each extract solution. A red-orange precipitate indicated a positive reaction. The appearance of white or white-yellow precipitate after addition of 2 drops of Mayer's reagent to 2 mL of each extract solution indicated the presence of alkaloids.

Flavonoids

In a tube containing 2 mL of extract, a few drops of 10% NaOH were added. Appearance of yellow-orange color indicated the presence of flavonoids. In the second method, two drops of ferricchloride (FeCl3) were added to 2 mL of extract. Appearance of greenish color reveals the presence of flavonoids.

Saponins

Samples (0.1 g of dry extract) were dissolved in 10 mL of distilled water. The samples were shaken vigorously up and down for 30-45 seconds and then left for 15 minutes.

The height of the foam was measured. Persistent foam for more than 1 cm high indicated the presence of saponins.

Tannins

Two milliliters of water and few drops of 1% ferricchloride were added to 1 mL of extract. The appearance of a blue, blue-black or black coloration indicated the presence of gallic tannins, the green or dark green coloration showed the presence of catechic tannins.

Cardiac glycosides

Two milliliters of chloroform were added to 1 mL of each extract solution. The appearance of a reddish-brown coloration after addition of 1 mL concentrated sulphuricacid revealed the presence of cardiac glycosides.

Results and Discussion

Antimycobacterial activity

In the present study, the *in vitro* antimycobacterial activity of the roots of *Carissa edulis* and *Sarcocepha luslatifolius*, and the leaves of *Vitex doniana* was examined against clinical pathogenic strains of *Mycobacterium tuberculosis*. The extracts were evaluated in a concentration series of 5,000 to 9.75 µg/mL, while isoniazid and rifampicin respectively with 64 to 0.12 µg/mL and 83 to 0.16 µg/mL concentrations. The wells were pink below a concentration of 1,250 µg/mL for the extract of *C. edulis*, 625 µg/mL for the extract of *S. latifolius* and 312.5 µg/mL for *V. doniana* extracts. For isoniazid and rifampicin, the microtiter wells were pink respectively below a concentration of 2 µg/mL and 5.2 µg/mL. All well streated with sterile distilled water (the vehicle) were pink, demonstrating no inhibitory role of the vehicle, by contrast, for sterile control, the wells were blue (Table 1). These observations allow us to determine the minimum inhibitory concentrations of the extracts and positive control drugs presented in Table 2.

Table.1 Colors obtained for test suspension in the wells after reincubation

| | Conc (µg/mL) | 5,000 | 2,500 | 1,250 | 625 | 312.5 | 156 | 78 | 39 | 19.5 | 9.75 |
|-----------------------|--------------|-------|-------|-------|------|-------|------|------|------|------|------|
| RECE | Color | blue | blue | blue | pink | pink | pink | pink | pink | pink | pink |
| RESL | Conc (µg/mL) | 5,000 | 2,500 | 1,250 | 625 | 312.5 | 156 | 78 | 39 | 19.5 | 9.75 |
| | Color | blue | blue | blue | blue | pink | pink | pink | pink | pink | pink |
| LEVD | Conc (µg/mL) | 5,000 | 2,500 | 1,250 | 625 | 312.5 | 156 | 78 | 39 | 19.5 | 9.75 |
| | Color | blue | blue | blue | blue | blue | pink | pink | pink | pink | pink |
| INH | Conc (µg/mL) | 64 | 32 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0.25 | 0.12 |
| | Color | blue | blue | blue | blue | blue | blue | pink | pink | pink | pink |
| RIF | Conc (µg/mL) | 83 | 41.5 | 20.8 | 10.4 | 5.2 | 2.6 | 1.3 | 0.65 | 0.32 | 0.16 |
| | Color | blue | blue | blue | blue | blue | pink | pink | pink | pink | pink |
| GC + SDW | Color | pink | pink | pink | pink | pink | pink | pink | pink | pink | pink |
| Sterilecontrol | Color | blue | blue | blue | blue | blue | blue | blue | blue | blue | blue |

RECE = Root extract of *C. edulis*, RESL = Root extract of *S. latifolius*, LEVD = Leavesextract of *V. doniana*, INH = isoniazid, RIF = rifampicin, GC + SDW = Growth control treatedwithsteriledistilled water, Conc = concentrations

Table.2 Minimum inhibitory concentration (MIC in $\mu\text{g/mL}$) values of hydro-ethanolic extracts of tested medicinal plants

| Plant species | Part used | CMI |
|---------------------------------|-----------|-------|
| <i>Carissa edulis</i> | Roots | 1,250 |
| <i>Vitex doniana</i> | Leaves | 312.5 |
| <i>Sarcocaphe luslatifolius</i> | Roots | 625 |
| Isoniazid (INH) | - | 2 |
| Rifampicin (RIF) | - | 5.2 |

Table.3 Phytochemical composition of the tested extracts

| Plant extracts | Saponins | Alkaloids | Flavonoids | Tannins | Cardiac glycosides |
|---------------------------------|----------|-----------|------------|---------|--------------------|
| <i>Carissa edulis</i> | - | + | + | + | + |
| <i>Sarcocaphe luslatifolius</i> | + | + | + | + | + |
| <i>Vitex doniana</i> | + | + | + | + | + |

The leaves from *V. doniana* displayed the best *in vitro* activity against the mycobacterial strains tested. The MIC were $312.5 \mu\text{g/mL}$ for *V. doniana*, $625 \mu\text{g/mL}$ for *S. latifolius*, and $1,250 \mu\text{g/mL}$ for *C. edulis*. Under these same experimental conditions isoniazid (INH) and rifampicin (RIF) gave respectively minimal inhibitory concentrations of $2 \mu\text{g/mL}$ and $5.2 \mu\text{g/mL}$ (Table 2).

Phytochemicals

The results of the phytochemicals screening are recorded in Table 3. The presence of alkaloids, flavonoids, tannins, saponins, and cardiac glycosides is revealed in the tested extracts except saponins which are absent in *Carissa edulis* roots extract.

Current TB therapy consists of treatment with a combination of drugs. This combination therapy causes hepatotoxicity as the major side effect as well as development of drug resistance (Kahaliw *et al.*, 2017). Using medicinal plants for the treatment of TB offers a great hope to fulfill these needs because of their chemical diversity and they

have been used for curing diseases for many centuries (Lawal *et al.*, 2011). In addition, natural herbs continue to play a great significant role in the drug discovery and development of highly active antimycobacterial metabolites and they can be used as pure compounds or as crude materials (Guzman *et al.*, 2010). In this study, the inhibitory activity of extracts on *Mycobacterium tuberculosis* was evaluated using the "Microplate Alamar Blue Assay (MABA)" study protocol. Crude hydroethanolic extracts of all the three plants tested for their antimycobacterial activity against *M. tuberculosis* strains by using MABA had showed antimycobacterial activity with the mean MIC values ranging from 312.5 to $1,250 \mu\text{g/mL}$. According to Kuete (2010), a crude extract is considered to have antimicrobial activity during a susceptibility test if its minimum inhibitory concentration (MIC) is in the range of 100 - $1,000 \mu\text{g/mL}$.

The activity is significant when the MIC is less than $100 \mu\text{g/mL}$, moderate when the MIC is between 100 - $625 \mu\text{g/mL}$ and low when

the MIC is higher than 625 µg/mL. Therefore, *V. doniana* leaves and *S. latifolius* extracts have moderate activity on mycobacterial growth while it is low for extracts from *Carissa edulis* roots. The antimycobacterial activity of the plants tested is very little studied. The study of Kahaliw *et al.*, (2017) has shown that the chloroform extract of the root of *C. edulis* is inactive on the *M. tuberculosis* H37Rv strain. But, the antimicrobial activity of the extracts of *C. edulis* has been demonstrated against *Escherichia coli* and *Staphylococcus aureus* (Abdu *et al.*, 2008). No report was found during a literature search against *Mycobacterium* strains apart from ethnobotanical reports on *S. latifolius* and *V. doniana*. Therefore, this investigation would be the first report on their antimycobacterial activities. As for *S. latifolius*, its extracts of stem bark and leaves have been tested for their antimicrobial activity while the activity of its root has not yet been elucidated (Djeussi *et al.*, 2016). Other studies have shown that extracts of *V. doniana* possess antimicrobial activities against *E. coli*, multidrug resistant *B. cepacia* and *P. aeruginosa*, methicillin-resistant *S. aureus*, and vancomycin-resistant Enterococcus (Taiwo *et al.*, 2009; Nwachukwu and Uzoeto, 2010). Studies have reported several pharmacological properties of these tested plants confirming their usefulness in traditional medicine (Amégbor *et al.*, 2012; Ogbole *et al.*, 2018; Mailu *et al.*, 2020).

The phytochemical study on the three plants showed the presence of alkaloids, flavonoids, tannins, saponins, and cardiac glycosides except the saponins which are absent in the extract of *C. edulis*. *C. edulis* extract does not contain saponins and is the one which gave the low activity among the extracts tested. This lets us say that the saponins may have played a role in the antimycobacterial activity of the extracts tested. Previous studies have

reported that antimycobacterial activity against *M. tuberculosis* maybe due to the bioactive constituents, such as flavonoids, saponins, tannins, alkaloids, phenols, and others (Arya, 2011; McCarthy and Mahony, 2011).

This report agrees with the previous of other authors who also found that the extracts of *V. doniana*, *C. edulis* and *S. latifolius* contain flavonoids, saponins, tannins, alkaloids, and others (Deleke Koko *et al.*, 2011; James *et al.*, 2014; Kaunda and Zhang, 2017).

In conclusion the hydroethanolic extracts from the leaves of *V. doniana* and roots of *S. latifolius* and *C. edulis* inhibited *in vitro* the growth of *Mycobacterium tuberculosis* and contain chemical constituents responsible for antimicrobial activities. The overall results of the present study provides baseline information for the possible use of the tested plants, especially *V. doniana* and *S. latifolius* in the control of infections due to *Mycobacterium tuberculosis*. But, these plants should be fractionated to obtain the most fractions with promising antimycobacterial activity and then, to conclude their anti-tuberculosis activity. In addition, further investigations should focus on isolating and identifying chemical constituents responsible for anti-TB activities observed in these plants and also elucidating the toxicity of the tested plants.

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