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Full Length Research Paper

Efficacy of *Cryptolepis sanguinolenta* root extract on slow-growing rifampicin resistant *Mycobacterium tuberculosis*

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We report here, the results from total crude methanol extract as well as serial ether, chloroform, and methanol extracts of the root of *Cryptolepis sanguinolenta* (Lindl) Schltr that were screened against three strains of *Mycobacterium tuberculosis.* The strains used included the pan sensitive H37Rv, the rifampicin-resistant TMC-331 and a wild strain of *Mycobacetrium avium* (MA) isolated from a Ugandan patient. The disc diffusion method was used for susceptibility tests on solid Middle brook 7H10 while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the microtitre plate method using Middle brook 7H9 broth. We report that the total crude methanol extract showed the highest activity against H37Rv and TM-331 with complete clearance of quadrants at 50 mg/ml and zones of inhibition of 10.0 to 11 mm at 25 mg/ml concentration although it was not effective against *M. avium*. The corresponding MIC values 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, while for rifampicin the MIC value was 0.25 µg/ml for H37Rv but it was not active on TMC-331. Acute toxicity test gave an LD_{50} of 758.5 mg/kg body weight while the phytochemical analysis showed the presence of alkaloids, tannins and flavonoids.

Key words: Anti-mycobacterial, *in vitro* activity, medicinal plant, *Cryptolepis sanguinolenta, Mycobacterium tuberculosis, Mycobacterium avium,* rifampicin, isoniazid.

INTRODUCTION

Tuberculosis (TB) remains a devastating global public health problem in developing countries. Approximately 9 million people are diagnosed with the disease of which 2 million people die annually (Sanjay, 2004; Navin et al., 2002). Globally, more than one-third of the world's population (about 2 billion) is infected with the bacterium that causes TB (WHO, 2006; CDC, 2005; Navin et al., 2002). The emergence of multi-drug resistant strains of *Mycobacterium tuberculosis* and more recently extensively drug resistant tuberculosis (XDR TB) poses a formidable challenge to the control of the disease (CDC,

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2002). Chemotherapy is the mainstay of tuberculosis control and there is need to develop new drugs for the control of tuberculosis, particularly the multi-drug resistant (MDR) and XDR TB strains. There is also need to alleviate the shortcomings of current drug regimens by developing safer, more effective and more affordable agents that can act over shorter periods of time.

Plants have been a source of effective chemotherapeutic agents for various infectious diseases and there is a growing interest in the development of drugs of plant origin. Many communities in East Africa, in general, and Uganda in particular, traditionally use plants to treat various infections, including respiratory tract infections. There are claims that some actually can treat tuberculosis.

This study was prompted by an ethno botanical survey carried out in 2007, to identify plants used to treat tuberculosis in the Lake Victoria Basin. A large number of medicinal plant species were reported as being used by traditional practitioners to treat TB in the Lake Victoria basin in Uganda, and *Cryptolepis sanguinolenta* (Lindl.) Schltr (Family: Periplocaceae, Synonym: Apocynaceae) was one of them. It is a slender climber up to 25 ft. high with greenish-yellow flowers and exists in many countries in sub-Saharan Africa including Uganda [(Aluka at: http://www.ithaka.org/), 2009].

Various uses of *C. sanguinolenta* have been reported; it is mainly used in herbal medicine as an antimalarial in West African ethnomedicine (Ansah and Gooderham, 2002) and the antimycobacterial activity of its alkaloid, cryptolepine, has been reported against fast growing mycobacterial species, *Mycobacterium fortuitum* (Gibbons et al., 2003). It is also reported to be a potential anticancer agent (Ansah and Gooderham, 2002). In addition to cryptolepine, a number of active alkaloids including neocryptolepine, biscryptolepine, cryptolepine and isocryptolepine, have been isolated from the root back extracts of the plant (Cimanga et al., 1996).

In this study, we investigated the activity of *C. sanguinolenta* against various strains of slow growing mycobacteria which also included a rifampicin-resistant strain. Acute toxicity and preliminary phytochemical tests were also done.

MATERIALS AND METHODS

Study design

This study was conducted using an experimental design. The roots of *C. sanguinolenta* (Kafulu in Luganda) were collected from Kayunga District. Crude extracts were then prepared tested in a bioassay on various strains of *M. tuberculosis*. Susceptibility tests and minimum inhibitory concentration (MIC) for the active extracts were determined. Acute toxicity tests for the most active extracts were also performed.

Selection criteria

The study plant was selected on the criterion that it had been mentioned by at least four traditional healers as being used in the treatment of tuberculosis (TB).

Plant collection and identification

Roots of *C. sanguinolenta* were harvested from Kayunga District in Central Uganda at 1207 m, 01°13'N32°52'E, by a team, which included a taxonomist, who identified the collected specimens. A voucher specimen (JRST 778) was prepared and deposited at the Makerere University Herbarium.

Drying and pulverizing

The plant parts were dried under shade to avoid direct sunshine that could degrade some of the compounds in the plants. They were also turned over regularly, to avoid fermenting and rotting. The dried parts were then pulverized using a plant mill, the powder weighed using an analytical scale, and stored at room temperature.

Extract preparation

A total crude methanol extract was prepared by soaking 500 g of the powder in 1000 ml of methanol for seven days. Then another 500 g plant powder was serially extracted by soaking in 1000 ml of ether, chloroform and lastly methanol following the order of polarity of the solvents for three days each, with occasional shaking. Whatman's filter paper No. 1 was used for filtering to obtain the crude extract solution. The crude solution was then concentrated to a minimum volume by a rotary evaporator (Büchi Labortechnik AG, Switzerland) at 40°C under reduced pressure. The conce ntrated crude extracts were then allowed to evaporate to constant weight at room temperature.

Mycobacterial tests

These tests were done in a Level III Safety Laboratory at the Joint Clinical Research Center (JCRC) in Mengo, Kampala, Uganda.

Mycobacterium strains used

Three preserved strains of Mycobacterium used, were obtained from Joint Clinical Research Centre (JCRC). They included a rifampicin-resistant strain (TMC -331strain) to serve as an indicator of MDR, a fully susceptible strain (H37Rv) as a control, and *Mycobacterium avium* (MA) a wild strain from a Ugandan patient to represent the *Mycobacterium* other than tuberculosis (MOTT) group.

Growth media

Middle brook 7H10 agar supplemented with oleic acid-albumin-

catalase (OADC) was used for reviving and culturing the mycobacteria for sensitivity testing (Parish and Stroker 1998). The medium was from Becton Dickinson Microbiology Systems of Becton Dickinson Company (Difco™), 7 Loveton Circle, Sparks, Maryland, USA; Lot No. 8175150. The OADC, Lot 8136781 also from Becton Dickinson Company. No adjustments for pH were made.

Preparation of inoculum for drug sensitivity testing

Preserved strains were revived on Middle brook 7H10 agar, prior to anti tubercular susceptibility testing. Colonies were scraped from freshly growing colonies (3 weeks old) on Middle brook 7H10 plates and introduced into 10 ml of saline. Bacterial suspensions with 0.5 McFarland standard turbidity equivalents to 10^8 CFU were prepared by dilution with saline.

The tubes were vigorously vortexed for 30 s in a glass bottle containing glass beads and the particles allowed to settle (Parish and Stroker, 1998).

Bioassay protocol for susceptibility tests

Preparation of the drugs/ extracts

The dried crude extracts (1 g) were each dissolved in methanol (20 ml) to a concentration of 50 mg/ml. Sterilization of the extracts was done using 0.2 µm single use filters. For rifampicin, a stock solution of 5.0 mg/ml was prepared by dissolving 0.1 g in 10 ml of methanol. A stock solution of 2.5 mg/ml of isoniazid was prepared by dissolving 0.1 g in 20 ml of distilled water.

Preparation of biodiscs

A volume of 20 µl for each of the drugs and extracts was used per disc, for the general susceptibility tests so that for the extracts each disc contained 1 mg of the extract; 0.05 mg/disc of isoniazid and 0.1 mg/disc for rifampicin. The discs were left to dry for 24 h. The culture medium was sterile Middle brook 7H10 agar placed in 90 mm diameter Petri dishes with quadrants. In each quadrant of the Petri dish was put 5.0 ml of the medium. The solidified medium in the quadrants was inoculated using a swab. A rifampicin-impregnated disc was placed in the first quadrant, in the second quadrant was put isoniazid impregnated disc the third quadrant had an extract impregnated disc while the fourth quadrant contained a blank disc as a negative control. All the tests for the extracts and the three strains of Mycobacteria were done in triplicate.

The Petri dishes were then left in the hood overnight, to allow diffusion of the extracts and drug and then sealed with a carbon dioxide-permeable tape. These were then incubated at 37℃ in a carbon dioxide incubator for up to four weeks.The sensitivity of *M. tuberculosis* and *M. avium* to the extracts and the drugs was determined by measuring the zones of inhibition surrounding the discs, using a transparent millimeter scale.

Determination of the minimum inhibitory concentration (MIC)

Microtitre plate method was used in the determination of MIC. Serial dilutions of the drugs/extracts were used to determine the minimum inhibitory concentration of the drug or extract, using Middle brook 7H9 from Becton Dickinson Microbiology Systems of Becton Dickinson Company (Difco™), 7 Loveton Circle, Sparks, Maryland, USA; Lot No. 5123072 as the medium. The OADC, Lot 8136781, was also from Becton Dickinson Company (Pauli et al., 2005; Parish and Stroker, 1998).

Procedure

The procedure followed was that described by Pauli et al. (2005) with some modifications. Middle brook 7H9 broth (100 µl) was dispensed into all the wells of a sterile 96-well microtitre plate. Then 100 µl of each of the drugs and extracts was added using a pipette. The drug/extract was mixed well with the medium, by sucking up and down six times and then serial doubling of dilution was made. This was done by successive transfers of 100 µl up to column 10, with the 100 µl from the well in column 10 being discarded instead of placing it into column 11. Using a micropipette, 5 µl of bacteria (10^4 to 10^5CFU/ml) were dispensed in columns 1 to 11 for all the rows containing the drugs and extract. The well in column 12 was left blank, as a sterility control. The solvent used to dissolve the drugs and extracts (methanol) was also titrated in one of the rows as a control for solvent. All the MIC tests were done in duplicate and the plates were then incubated at 37℃ for up to four weeks. The lowest concentration with no visible turbidity was taken to be the MIC.

Acute toxicity tests

Acute toxicity tests on the most active extract were carried out on mice, *Mus musculus.* It was done as described by Ghosh (1984) with few modifications. Only the total crude was tested. Mice of both sexes, for each concentration were used and they were fasted overnight before giving them the extracts orally using a gastro intestinal tube. A pilot study was carried out on pairs of mice, using widely separated doses of 50, 200, 500, 800 and 1000 mg/kg to determine approximate lethal and non-lethal dose ranges. Five different groups of 5 mice each were used in the experiment given in doses of 700, 750, 800, 850 and 900 mg/kg body weight as a single dose. The control groups were given DMSO. Observations were made and recorded after 24 h. LD_{50} was then determined plotting percentage mortality against log dose graph.

Qualitative phytochemical testing

In the most active extracts qualitative tests for terpenoids, saponins, tanins, flavones, phenols and alkaloids were carried out as described by Edeoga et al. (2005).

Test for terpenoids

To the extract (1 ml) chloroform was added and then an equal volume of concentrated sulphuric acid was added. Formation of a bluish red coloration indicated presence of terpenoids.

Test for tannins

The dried powdered extract (0.5 g) was boiled with water (20 ml) in

Table 1. The antimycobacterial activity of ether, methanol, chloroform and total crude extracts of *C. sanguinolenta* at 50 mg/ml using the biodisc diffusion method.

Extract/Strain	Zones of inhibition ± standard deviation		
	H37RV	TMC 331	MA
CKVT (a)	Cleared	Cleared	6.0± 0
CKVT (b)[1]	10.0±1.0	11.0±1.0	6.0±0
CKVE	6.0±0.0	6.0±0.0	ND
CKVC	6.0±0.0	6.6±0.56	ND
CKVM	10.6±0.56	6.0±0.0	ND
Isoniazid[2]	Cleared	Cleared	6.0±0
Rifampicin[3]	Cleared	6.0±0	6.0±0
Negative control	6.0±0	6.0±0	6.0±0

Key: CKVT = *Cryptolepis sanguinolenta* total cude extract, CKVE = *C sanguinolenta* ether extract, CKVC = *C sanguinolenta* chloroform extract, CKVM = *C sanguinolenta* methanol extract. [1]The concentration of extract used was 25 mg/ml instead of 50 mg/ml used in case VT10(a). [2]Concentration of isoniazid used was 2.5 mg/ml. [3]Concentration of rifampicin used was 5 mg/ml.

a test tube and hen filtered. Few drops of 0.1 M iron III chloride ($FeCl_3$) were added. Formation of a blue-black coloration indicated presence of tannins.

Test for flavones

Ammonium solution (5 ml) was added to a portion of aqueous filtrate of the extract followed by addition of sulphuric acid. A yellow coloration indicated presence of flavones.

Test for phenols

Iron III chloride (2 ml) was added to the extract (2 ml). Formation of a deep bluish solution indicated presence of phenols.

Test for alkaloids

About 50 g of the powder was mixed with 250 ml of 1% sulphuric acid. It was allowed to stand and then filtered. 10 mls of the filtrate was shaken and added to Meyer's reagent. Formation of a white precipitate indicated presence of alkaloids.

Test for saponins

The powdered sample was boiled in distilled water (20 ml) and filtered. The filtrate (10 ml) was mixed with distilled water (5 ml) and shaken vigorously. Formation of a persistent froth indicated presence of saponins.

Data collection and analysis

The numerical data from the replicated investigations is presented in form of tables. Statistical analysis involved use of the statistics computer program, SigmaPlot, New Version 10, of Systat Software Inc. (2002) and Graph pad prism version.

Ethical considerations

Ethical approval was sought from the Research and Ethics Committee of the Faculty of Medicine and the Uganda National Council for Science and Technology. Protection of the investigators was ensured by carrying out the work in collaboration with, and under the guidance of the Mycobacteriology Laboratory staff at the Joint Clinical Research Centre, Mengo in Kampala, who had the necessary knowledge for handling *M. tuberculosis*. Also the necessary protective wears including respirators and gloves as well as safety cabinets were used, to minimize the risk of exposure to *M. tuberculosis*. Guidelines for the handling of Laboratory animals were followed. Animals were sacrificed under general anesthesia (Ghosh, 1984).

RESULTS

Antimycobacterial activity

Results of susceptibility tests

The susceptibility tests were done in triplicate and the mean of the three values and standard deviation are presented in Table 1. The zones of inhibition also include the diameter of the disc, which was 6 mm. Rifampicin was not active on *M. avium* complex and the rifampicin-resistant strain, TMC-331 but showed a zone of inhibition of 26 mm for H37Rv (a pan sensitive strain) at a concentration of 0.1 mg. Isoniazid cleared the quadrant for both H37Rv and TMC-331 at a concentration of 0.05 mg but was not effective on *M. avium* . Results for MIC:

Table 2. The minimum inhibitory inhibition of ether, methanol, chloroform and total crude extracts of *C. sanguinolenta* using the microtitre method.

Extract/Strain	Minimum inhibitory concentration ± standard deviation		
	H37RV	TMC- 331	MA
CKVT	3.13±0.0	3.13±0.0	ND
Rifampicin	0.5±0.0	-	-
Isoniazid	0.25±0.0	0.25±0.0	-

Table 3. Behavioral changes made during acute toxicity studies.

Characteristic	CKVT
↑sed motor activity	-
Tremors	+
Pilo erection	-
↓sed motor activity	+
Sedation	+
Analgesia	-
Lacrimation	-
Diarrhea	-

From Table 2, the total crude extract of *C. sanguiolenta* had an MIC of 1.17 and 1.56 mg/ml against H37Rv and TM-331, respectively while for rifampicin the value was 0.25 µg/ml against H37Rv and for isoniazid it was 0.25 and 9.38 µg/ml *against* H37rv and TMC-331, respectively.

Results of acute toxicity tests: Table 3 shows behavioral changes that were observed during the first six hours after drug administration, four observations were made for the mice that were administered with *C. sanguinolenta* total crude extract.

These were decreased motility, sedation, frequent urination and finally tremors. Other observations like diarrhea, analgesia, pilo-erection were not observed. The control group was given DMSO and no peculiar changes in behavior were observed.

Table 4 shows results obtained after a single dose administration of the total crude extract of *C. sanguinolenta* to *M. musculus*. The doses that were given were estimated from a preliminary study. The number of mice found dead after 24 h was thereafter recorded as shown earlier. No mice died in the first six hours of drug administration but during preliminary studies mice that were administered with a dose of 1000 mg/kg died after four hours. From a plot of the percentage mortality of mice against log dose administered, the LD_{50} of the crude extract was found to correspond to a log dose of 2.98 which is 758.5 mg/kg.

DISCUSSION

The total crude methanol extract of *Cryptolepis sanguinolenta* was active on two strains that included the pan sensitive strain (H37Rv) and the rifampicin resistant strain (TMC-331). However, the potency of the extract was much lower, compared to the standard drugs used. This could be attributed to the fact that isoniazid and rifampicin were in a pure state while the extract was just crude.

If the compound is isolated and purified it, could show a comparable activity or even more interestingly have a synergistic effect with the pure drugs. Nevertheless, *C. sanguinolenta* showed advantage over rifampicin by being active on the rifampicin-resistant strain of *M. tuberculosis.* This makes it a potential source of a lead compound that could be developed into a drug to tackle the problems of multi-drug resistance, since resistance to rifampicin is a good indicator of multi-drug resistance (MDR). This is especially important since the acute toxicity studies carried out on the total crude extracts of *C. sanguinolenta* revealed that the two extract had an LD_{50} of above 500 mg/kg body weight, which according to Gosh (1984) could be considered relatively safe. The

Table 4. Acute toxicity of *Cryptolepis sanguinolenta* total crude extract.

Dose (mg/kg)	Log dose	No. of mice dead	No. of mice alive	% Death
700	2.845	1	4	20
750	2.875	2	3	40
800	2.903	3	2	60
850	2.929	3	2	60
900	2.954	4	1	80

behavioral changes recorded that included sedation and decreased motor activity at doses higher than 500 mg/kg body weight could be central nervous system (CNS)-related. *C. sanguinolenta*-treated mice showed signs of tremors and frequent urination at very high doses (1000 mg/kg). This could mean that the plant could have some diuretic effects or could be a result of effects on the autonomic nervous system. It is hoped that isolation of active compounds and subsequent *in vivo* screening could reveal the actual sites of pharmacological action of this extract.

Phytochemical tests of the *C. sanguinolenta* total crude methanol extract showed presence of alkaloids, tannins and flavones. On the contrary, tannins and flavonoids were not identified in the root bark of *C. sanguinolenta* in a study done by Tona et al. (1998). This may be attributed to the different methods of preparation of the crude extracts.

Previous studies done on the antimycobacterial activity of the renowned antimalarial climbing liana *C. sanguinolenta* by Gibbons et al. (2003) are in agreement with the findings of this study though activity was on fast growing non-virulent mycobacteria. This is therefore a confirmation that the plant extract, besides being active on the fast growing mycobacteria strains, it is also active on the slow growing virulent strains of mycobacteria that were used in this study. Gibbons et al. (2003) went ahead to isolate an alkaloid (cryptolepine), which was responsible for the activity. This explains why the minimum inhibitory concentrations of *C. sanguinolenta* in this study are much higher than those in the previous study. Presence of alkaloids in *C. sanguinolenta* is in agreement with the simple phytochemical tests done on the plant in this study.

Though saponins and flavones have been identified in *C. sanguinolenta* total crude extract, the compound which could have caused the antimycobacterial activity could have been the cryptolepine alkaloid, however, more studies are needed to confirm this (Gibbons et al., 2003). Acute toxicity studies on *C. sanguinolenta* in this study were contrary to what was got by Ansah et al. (2009) where the LD_{50} was found to be about 3000 mg/kg. The difference in the values could be attributed to the fact that they used rats, which could have been more tolerant to the extract compared to the mice used in this study.

Furthermore, water extracts were tested in their study while the methanol extract was used in this study. It is possible that the methanol extract contained more pharmacologically active compounds than the water considering the fact that non-polar compounds are not readily soluble in water. However, they agree that there was prolonged sleep in the animals and also that there could be some CNS disturbances which were observed as tremors, sedation and decreased motility in this study. A study done by Ansah et al. (2008) also concluded that *C. sanguinolenta* could synergize with hypno-sedatives or other CNS depressants and therefore caution needs to be taken in the concomitant administration of the plant with other CNS depressants. Ansah et al. (2009) also agree that the extract is safe for use at doses less than 500 mg/kg. Furthermore, *in vitro* studies have reported cytotoxicity at the molecular level although this may not be reflected *in vivo* (Ansah and Gooderham, 2002).

The results of this study have further shown that the search for new bioactive products using the ethno botanical criterion increases chances of finding active compounds that could be used for new drug development against TB (Newton et al., 2000).

Conclusion

C. sanguinolenta has reasonable antimycobacterial activity and is relatively safe. This therefore gives credence for their use in the treatment of tuberculosis by traditional practitioners.

However, isolation and screening active compounds and more *in vitro* and *in vivo* studies on the toxicity of the plants are needed before declaring them completely safe for use in humans.

Limitations

The results from acute toxicity tests have shortcomings in

that they measure mortality and sub lethal toxicity. Also, the tests measure only acute toxicity produced by a single dose, and not long-term toxicity and therefore, cannot measure idiosyncratic reactions though such reactions may be more relevant in practice than high dose toxicity.

Recommendations

The active compounds of the plants should be isolated and identified. It is also recommended that, some tests be done to find out whether these compounds could have a synergistic effect on each other and whether they are active on the resistant strains of TB.

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REFERENCES

Ansah C, Mfoafo EA, Woode E, Opoku-Okrah C, Owiredu WKBA (2008). Toxicological Evaluation of the Anti-Malarial Herb *Cryptolepis sanguinolenta* in Rodents. J. Pharmacol. Toxicol., 5: 335-343.

Ansah C, Gooderham NJ (2002). The popular herbal antimalarial extract *Cryptoleps sanguinolenta* is potentially cytotoxic. Toxicol. Sci., 70: 245-251.

Ansah C, Otsyina R, Duwiejua M, Woode E, Aboagye FA, Aning KG. (2009). Toxicological assessment of *Cryptolepis sanguinolenta* for possible use in veterinary medicine. J. Vet. Med. Anim. Health, 1(1): 011-016.

Centres for Disease Control (2005). Worldwide emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs. MMWR Morb. Mortal. Wkly Rep., 55: 250-253.

Cimanga K, De Bruyne T, Pieters L, Claeys M, Vlietinck (1996). New alkaloids from *Cryptolepis sanguinolenta.* Tetrahedron Lett., 37: 1703-1706.

Cryptolepis sanguinolenta (Lindl.) Schltr. [Family: ASCLEPIADACEAE] Aluka at: http://www.ithaka.org/http://www.ithaka.org/) (2009). Accessed 15th January, 2010.

Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4: 685-688.

Ghosh MN (1984). Fundamentals of Experimental Pharmacology. 2nd Edition. Scientific Book Agency, Calcutta, 1-11; 153-190.

Gibbons S, Fallah F, Wright CW (2003). Cryptolepine Hydrochloride: A potent antimycobacterial alkaloid derived from *Cryptolepis sanguinolenta.* Phytother. Res., 17(4): 434-436.

Navin TR, Mc Nab SJ, Crawford JT (2002). The continued threat to tuberculosis. Emerg. Infect. Dis., 8: 1187.

Parish T, Stroker NG (1998). Mycobacteria Protocols: Methods in molecular Biology. (Vol. 101) (eds,) Humana Press, Totowa, NJ. Methods Mol. Biol., 101: 395-422.

Pauli GF, Case RJ, Inuli T, Wang Y, Cho S, Fischer NH, Franzblau SG (2005). New perspectives on natural products in TB drug research. Life Sci., 78: 485-494.

Sanjay MJ (2004). Natural Products: An important source for Anti-tubercular Drugs. CRISP., 5: 1.

Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ (1998). Antiamoebic and phytochemical screening of some Congolese medicinal plants. J. Ethnopharmacol., 61: 57-61.

World Health Oorganization (2006). Global Tuberculosis Database. [Online Tuberculosis Database as of 21st March 2005].