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# Antimycobacterial, antibacterial and antifungal activities of the methanol extract and compounds from *Thecacoris annobonae* (Euphorbiaceae)

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**Abstract**

This study was designed to evaluate the antimycobacterial, antibacterial and antifungal activities of the methanol extract from the stem bark of *Thecacoris annobonae* Pax & K. Hoffm, that of aristolochic acid I (**1**) and other isolated compounds. The microplate alamar blue assay (MABA) and the broth microdilution method were used to determine the minimal inhibitory concentration (MIC) and minimal microbicidal concentration (MMC) of the above samples. The $H^+$-ATPase-mediated proton pumping assay was used to evaluate a possible mechanism of action for both the methanol extract and aristolochic acid I. The results of the MIC determinations showed that the methanol extract and aristolochic acid I prevent the growth of all studied organisms. The results obtained in this study also showed that the methanol extract as well as aristolochic acid I inhibited the $H^+$-ATPase activity. The overall results provided evidence that the methanol extract of *T. annobonae* might be a potential source of new antimicrobial drug against tuberculosis, and some bacterial and fungal diseases, but should be consumed with caution, bearing in mind that the main active component, aristolochic acid I is a potentially toxic compound.
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*Keywords:* Antimicrobial activity; Aristolochic acid I; Compounds; Euphorbiaceae; Methanol extract; *Thecacoris annobonae*

## 1. Introduction

Infectious diseases are serious health problems worldwide. Tuberculosis (TB) infection, caused by *Mycobacterium* species remains one of the most important modifiable infectious human diseases in the developing world with more than 2 billion people being infected (McGaw et al., 2008). World statistics of TB reached a ceiling of estimated 9.2 million new cases and 1.7 million deaths in 2006 (McGaw et al., 2008). TB is widespread in poor countries with the highest incidence of the disease (more than 80% of cases) occurring in Asia and Africa (Zager and McNerney, 2008). The annual incidence of the disease indicates a rate of over 600 cases per 100,000 reported in many sub-Saharan Africa countries (Corbett et al., 2006) with the persistent increase attributed to the acquired immune deficiency syndrome pandemic combined with inadequate healthcare systems (Zager and McNerney, 2008). Typhoid fever (TF) is a more classical systemic infection caused by the typhoid bacillus, *Salmonella enterica serovar* Typhi, the most common cause of enteric fever, which also includes paratyphoid fever caused by *S. paratyphi* A, B and C. With an estimated 16–33 million cases resulting in 500,000 to 600,000 deaths annually in endemic areas, the WHO (2009) identifies typhoid as a serious public health problem. The true burden of TF in developing countries is difficult to estimate. According to recent estimates, 22 million cases occur each year causing 216,000 deaths, predominantly in school-age children and young adults (Crump et al., 2004). Asia, with 274 cases per 100,000 persons has the highest incidence of TF cases worldwide, especially in

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Southeast Asian countries and on the Indian subcontinent, followed by sub-Saharan Africa and Latin America with 50 cases per 100,000 persons. In an urban slum in Dhaka, incidence of TF was found to be 390/100,000 persons (Brooks et al., 2005). *Staphylococcus aureus* can cause a range of illnesses from minor skin infections to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, and septicemia (Carbonnelle et al., 1987). *Pseudomonas aeruginosa* is a highly relevant opportunistic pathogen and one of its most worrisome characteristics is its low antibiotic susceptibly (Sleigh and Timbury, 1998). Also, *Candida* species are the predominant pathogens causing invasive disease in intensive care, invasive fungal infections being of increasing relevance for severely ill and immuno-compromised patients (Presterl et al., 2009), with up to 50% mortality (Wenzel, 1995).

Due to the permanent resistance of the microorganisms to available drugs, continuous search for new antimicrobials is a scientific challenge. Natural products continue to play a most significant role in the drug discovery and development process (Newman and Cragg, 2007), and plants are recognized as a useful sources of highly active antimicrobial metabolites (Gibbons, 2005; Pauli et al., 2005). In our continuous search of antimicrobial agents from natural sources, this study was designed to assess the antimycobacterial, antibacterial and antifungal activities of *Thecacoris annobonae* Pax & K. Hoffm (Euphorbiaceae). *T. annobonae* is a tree found in Cameroon and Equatorial Guinea. Its natural habitat is subtropical or tropical moist lowland forests and was listed as an endangered species (Cheek, 2004). Though few medicinal properties have been reported on the genus *Thecacoris*, *T. batesii* is used as a purgative and antirheumatic remedy in the traditional medicine in Cameroon (Ngadjui and Abegaz, 2003; Ngadjui et al., 2007).

## 2. Material and methods

### 2.1. Plant material, instruments, solvents, extraction and isolation

The stem bark of *T. annobonae* was collected in March 2005 at Kumba, South-West (Cameroon) and identified at the Cameroon National Herbarium, where a voucher specimen is deposited (Ref. No 38569/HNC).

Air dried powdered stem bark of *T. annobonae* (2.8 kg) was extracted with MeOH at room temperature for 48 h. After removing the solvent by evaporation under reduced pressure, the crude extract (100.2 g) was chromatographed on silica gel using hexane/ethyl acetate in increasing polarity (pure hexane; hexane/ethyl acetate 7.5/2.5; hexane/ethyl acetate 5:5; hexane/ethyl acetate 2.5/7.5; pure ethyl acetate). One hundred and fifteen fractions were collected and pooled on the basis of analytical thin layer chromatography in three main fractions, A (fraction 1 to 36); B (37–88); and C (89–115).

Fraction A (5.0 g) was column chromatographed using silica gel 60 and eluted with $CH_2Cl_2$–MeOH (1:3). Thirty-two sub-fractions of 100 ml each were collected and subtractions 13–26 were purified by using column chromatography on Sephadex LH-20 with $CH_2Cl_2$–MeOH (1:1) as eluent to afford aristolochic acid I $C_{17}H_{11}NO_7$ [**1**; yellow amorphous powder, 4.7 mg; $R_f$: 0.3 ($CH_2Cl_2$/3%MeOH); *m/z*: 341] (Priestap, 1989; Arlt et al., 2002) and aristolochic acid I methyl ester $C_{18}H_{13}NO_7$ [**2**; Yellow amorphous powder; $R_f$: 0.4 ($CH_2Cl_2$/3%MeOH); 9.0 mg; *m/z*: 355] (Arlt et al., 2002).

Fraction B (13.0 g) was chromatographed on silica gel and eluted with a mixture of hexane/ethyl acetate in increasing polarity. Fraction eluted hexane/ethyl acetate 40:60 yielded vanillic acid $C_8H_8O_4$ [**3**, Brown amorphous powder; 6.7 mg; $R_f$=0.3 ($CH_2Cl_2$/5% MeOH); *m/z*: 168] (Fang et al., 2008) and 4-acetoxy vanillic acid $C_{10}H_{10}O_5$ [**4**, amorphous powder; 10.0 mg; $R_f$: 0.5 ($CH_2Cl_2$/5% MeOH); *m/z*: 209] (Li and Huang, 2000).

Fraction C (17.0 g) was chromatographed on silica gel and eluted using hexane/ethyl acetate. A total of 50 fractions of 300 ml each were collected and combined on the basis of TLC analysis leading to two main series (I and II). Series I (1.5 g) [fractions 1–37] was column chromatographed on silica gel and eluted with $CH_2Cl_2$–MeOH (3:1) to yield friedelin $C_{30}H_{50}O$ [**6**, White powder; $R_f$: 0.7 ($CH_2Cl_2$); 3.9 mg; m.p.: 247–249 °C; *m/z*: 426] (Ageta and Arai, 1990). Series II (0.9 g) [fractions 38–50] eluted with $CH_2Cl_2$–MeOH (7:9) yielded friedelin-3β-ol $C_{30}H_{52}O$ [**5**, White powder; $R_f$: 0.6 ($CH_2Cl_2$); 5.4 mg; m.p.: 229–231 °C; *m/z*: 428] (Fun et al., 2007; Ng'ang'a et al., 2008).

### 2.2. General experimental instruments and procedure

Nuclear magnetic resonance (NMR) spectra were measured on Varian Unity 300 (300.145 MHz) and Varian Inova 500 (499.876 MHz) spectrometers. ESI mass spectra were recorded on A Finnigan LCQ with quaternary pump Rheos 4000 (Flux Instrument). ESI HR mass spectra were recorded on A Bruker FTICR 4.7 T mass spectrometer. EI mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance for HREI-MS. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer from films. Melting and decomposition points were measured with an Electrothermal (Yanaco, Tokyo-Japan) melting point apparatus and were not corrected.

### 2.3. Microbial strains and culture media

The test organisms included Mycobacteria namely *Mycobacterium smegmatis* ATCC 700084, drug-susceptible strain of *Mycobacterium tuberculosis* H37Rv ATCC 27294 (America Type Culture Collection, Rockville, MD, USA), two clinical strains, *M. tuberculosis* MTCS1, and *M. tuberculosis* MTCS2, Gram-positive bacteria (B) including a methicillin-resistant *S. aureus* B845, *Streptococcus faecalis* B 846, *Bacillus cereus* B864, Gram-negative bacteria namely β-lactamase positive ($\beta L^+$) *Escherichia coli* B831, ampicillin-resistant *Klebsiella pneumoniae* B837, carbenicillin-resistant *P. aeruginosa* B832, chloramphenicol-resistant *Salmonella typhi* B839, chloramphenicol-resistant *Citrobacter freundii* B828 and two fungi (F) namely *Candida albicans* F702 and *Microsporum audouinii* F712. The clinical isolates were obtained from Yaoundé General Hospital (Cameroon). Their identifications were confirmed (and they were encoded) before use at the Laboratory of Applied Microbiology

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