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## Potential of Isoniazid Efficacy against Isoniazid-resistant Mycobacteria Strains

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### ABSTRACT

This study aimed to isolate antimycobacterial compounds from the leaves of *Hallea rubrostipulata*, a medicinal plant used in the treatment of respiratory tract infection in Karagwe district, Tanzania. It further aimed to investigate the ability of compounds to break and/or circumvent the resistance of *Mycobacterium madagascariense* (MM) and *Mycobacterium indicus pranii* (MIP) against isoniazid. The isolation of compounds from the leaves extracts of *H. rubrostipulata* was achieved using various chromatographic techniques. Two indole alkaloids namely; Mitraphylline and isomitraphylline were isolated and their structures were deduced using Nuclear Magnetic Resonance (NMR) and Mass Spectrophotometry (MS) analyses. The antimycobacterial activity of indole alkaloids against isoniazid resistant strains namely; *Mycobacterium madascariense* (MM) and *Mycobacterium indicus pranii* (MIP) was evaluated using two folds broth microdilution method. The drug combination assay was done by blending  $\frac{1}{2}$  to  $\frac{1}{16}$  of alkaloid's MIC values with those of isoniazid (recorded previously against *M. tuberculosis*, strain Mtb H37Rv). The potential cytotoxicity activity of alkaloids was evaluated using Brine Shrimp Toxicity assay (BST). The indole alkaloids exhibited moderate antimycobacterial activity against test organisms. Mitraphylline had MIC values of 0.8 and 0.4 mg mL<sup>-1</sup> against MM and MIP, respectively, while isomitraphylline had MIC values of 0.8 mg mL<sup>-1</sup> against all organisms. In the drug combination assay, all compounds potentiated the activity of isoniazid against the two mycobacteria strains. The cytotoxicity assay revealed that, the two indole alkaloids are not toxic to shrimps. These results confirmed why *H. rubrostipulata* has for many years been used in the treatment of respiratory tract infections.

**Key words:** *Hallea rubrostipulata*, alkaloids, isoniazid, antimycobacterial activity, *Mycobacterium madagascariense*, *Mycobacterium indicus pranii*, drug combination

### INTRODUCTION

The infection with *Mycobacterium tuberculosis* (Mtb) and *Mycobacterium africanum* claim millions of human lives in the world. The two mycobacteria are responsible for tuberculosis (TB) infection in human, which is one of the world's health challenges. They affect over one third of the global population, mostly from developing countries (McKinney, 2000; WHO, 2005; Hugo *et al.*, 2009). Despite the availability of effective drugs like isoniazid and rifampicin, TB continues to be

an important cause of mortality worldwide (WHO, 2005). The severity of this disease is aggravated by the emergency of TB strains resistant to isoniazid and rifampicin, the front line antitubercular drugs (Lall and Meyer, 2001; O'Donnell *et al.*, 2006; Ouellet *et al.*, 2008). Therefore, new drug strategies are needed to combat the rising incidences of TB complications and attempt to shorten the treatment duration. One of the possible strategies is to screen alkaloids from medicinal plants and combinations of alkaloids with TB drugs like isoniazid.

Medicinal plants are an invaluable source of bioactive natural products which may be useful in the development of anti-TB drug combinations. They offer a wide range of chemical compounds with diverse chemical structures and biological activities. Among the compounds of interest are indole alkaloids. Plants species from the genus *Hallea* (Syn: *Mitragyna*) are known to be rich in indole alkaloids (Shellard *et al.*, 1969). One of those species is *Hallea rubrostipulata* (Rubiaceae) which is used in the treatment of various parasitic and microbial infections including respiratory tract infections in Karagwe District, Tanzania. The leaves of this species have been reported to be useful in the treatment of TB related infections in Uganda (Taniguchi *et al.*, 1978). The ethnomedical uses especially; the treatment of respiratory tract infections and TB related ailments have never been investigated. This study therefore reports the antimycobacterial activity of indole alkaloids isolated from the leaves and stem bark of *Hallea rubrostipulata* and their ability to potentiate the activity of isoniazid against isoniazid-resistant mycobacteria strains. *Mycobacterium madagascariense* and *M. indicus pranii* are two fast growing mycobacteria strains known to be highly resistant to isoniazid (INH) (Kazda *et al.*, 1992; Saini *et al.*, 2009). Because of their resistance to INH even at higher concentration, the two strains were chosen for use in the INH potentiation experiment. The combination of indole alkaloids and isoniazid with the aim to break INH resistance of *Mycobacterium madagascariense* and *M. indicus pranii* is reported here for the first time. The cytotoxicity evaluation of the two indole alkaloids against shrimps is also reported here in this study.

## MATERIALS AND METHODS

**General analyses:** The 1-D and 2-D NMR data of the isolated compounds were obtained using Bruker Avance Ultrashield 400 Plus NMR machine operating at a spectrometer frequency of 400 MHz for <sup>1</sup>H-NMR and 75 MHz for <sup>13</sup>C-NMR. Melting points were measured on a Stuart Scientific (SMP1) melting point apparatus.

**Chemicals:** All solvents were purchased from Carlo Erba (France), Middlebrook 7H9 broth base was obtained from HIMEDIA (India), Glycerol (AR) obtained from Lab Equip Ltd (Tanzania), iodinitrotetrazolium (INT) chloride, Ciprofloxacin and Isoniazid (RandD) were purchased from Sigma (UK), Cyclophosphamide was purchased from Sigma Aldrich (South Africa). Ninety six wells microtitre plates supplied by KAS Medics (Tanzania). Silica gel Kiesegel 60 PF<sub>254</sub> obtained from Merck South Africa Pty. Pre-coated aluminum backed silica gel 60 F<sub>254</sub> (0.2 mm thickness) TLC plates were obtained from Merck UK.

**Collection of plant materials:** Plant materials were collected from Karagwe District in Kagera Region, Tanzania. Identification was done on site by a plant taxonomist Mr Boniface Mhoro. Voucher specimen ITMHCM01 is deposited in the Herbarium of the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences. The permission to conduct a field

work was sought from Mr Rwebogora Mukombozi a farm owner where leaves and stem barks of *Hallea rubrostipulata* were collected. This is not an endangered species in Karagwe, although it is highly protected by farm owners for various medicinal purposes.

**Preparation and extraction of plant materials:** Plant materials were air dried under shade for two weeks and thereafter pulverized into powder using electric miller. The powdered leaves and stem barks of *Hallea rubrostipulata* were separately soaked in dichloromethane (DCM) three times each for 24 h to afford 50.8 and 23.7 g of crude extracts, respectively. The extracts were kept in the refrigerator (8°C) ready for chromatographic isolation of alkaloids.

**Isolation of alkaloids from the stem bark of *H. rubrostipulata*:** The dichloromethane extract (15.6 g) from the stem bark of *H. rubrostipulata* was adsorbed in silica gel and loaded on a silica gel column eluting with acetone:dichloromethane with increasing polarity from 0.3:9.7 to 1:9 and later 1.5:8.5, respectively. A total of 35 fractions each with 150 mL of eluates were collected. After TLC analysis, fractions 1-7, 8-18, 19-29 and 30-35 were combined. Of the combined fractions, only 19/29 contained alkaloids and therefore was subjected on further column chromatographic analysis which eluted with acetone:dichloromethane (0.3:9.7) to give 68 subfractions. The subfractions 1-14 were discarded, subfractions 15-28 were combined and loaded on a silica gel column eluting with acetone:dichloromethane (0.3:9.7) to yield 263.3 mg of clean yellow solids identified as compound 2. Fractions 30/51 yielded white needle-like crystals that were recrystallized in MeOH yielding 456 mg of compound 1. A portion of crystals 1 and 2 were dissolved in dichloromethane and spotted on TLC plate. The plate was developed in 9:1 (v/v) dichloromethane/petroleum ether. After drying, a TLC plate was sprayed by Dragendorff reagent to give strong orange spots against yellow background. This not only confirmed the purity of the compounds but also the class of compounds obtained as alkaloids.

**Isolation of alkaloids from the leaves of *H. rubrostipulata*:** Weighed 20 g of dichloromethane extract of the leaves of *H. rubrostipulata* was adsorbed in silica gel and loaded on silica gel column eluting with acetone: dichloromethane with increasing polarity from 0.3:9.7 to 2:8. A total of 32 fractions each with 150 mL of eluates was collected out of which fraction 3 and 4 yielded white crystals. The crystals were collected and recrystallized in methanol to yield compound 1 (137.9 mg). Fractions 5 to 8 were combined and subjected on silica gel column eluting with petroleum ether: dichloromethane 2:8 (10 subfractions), later adjusted to 3:7 (10 subfractions) and finally with 100% dichloromethane (10 subfractions). This gave 30 subfractions each with 100 mL of eluates. Subfractions 5 to 8 were combined and coded HR 5/10 and left to stand overnight where white needle crystals formed. The crystals were recrystallized in methanol to yield 152.1 mg of white crystals later identified as compound 1. A small portion of crystals was dissolved in dichloromethane and spotted on TLC to confirm the purity. The TLC was developed in 9:1 (v/v) dichloromethane/petroleum ether, after drying; the TLC was sprayed by Dragendorff reagent to give strong orange spots against yellow background. This confirmed that the crystals were a pure alkaloid. Further work on the remaining fractions 10/22 yielded white crystals weighing 308 mg of the same compound 1. The remaining subfractions were discarded as they did show interesting spots on TLC analysis.

**Mitraphylline (1):** White needle crystals, mp 264°C, <sup>13</sup>C-NMR data (Bruker Avance Ultrashield 400 MHz): δ 167.1 (C-1), 74 (C-3), 54.3 (C-5), 35.2 (C-6), 55.6 (C-7), 133.3 (C-8), 122.9 (C-9), 122.6 (C-10), 128.1 (C-11), 109.9 (C-12), 106.9 (C-13), 28.4 (C-14), 30.5 (C-15), 140.9 (C-16), 154.1 (C-17), 14.9 (C-18), 73.8 (C-19), 40.5 (C-20), 54.3 (C-21), 181.4 (C-22), 50.8 (C-23).

**Isomitraphylline (2):** Yellow solids, mp 110°C, <sup>13</sup>C-NMR data (Bruker Avance Ultrashield 400 MHz). δ 167.1 (C-2), 71.8 (C-3), 53.4 (C-5), 35.5 (C-6), 56.4 (C-7), 133.8 (C-8), 124.9 (C-9), 122.4 (C-10), 127.6 (C-11), 109 (C-12), 107.3 (C-13), 35.5 (C-14), 30.1 (C-15), 140.2 (C-16), 153.9 (C-17), 14.9 (C-18), 74 (C-19), 29.1 (C-20), 54.3 (C-21), 181.3 (C-22), 50.8 (C-23).

### Antimycobacterial screening

**Test organisms:** The mycobacteria strains, namely *Mycobacterium madagascariense* (MM) DSM 44641 and *Mycobacterium indicus* Pranii (MIP) DSM 45239 supplied by the Germany Resource Centre for Biological Materials, Braunschweig, Germany. The two acid-fast growing mycobacteria strains are isoniazid resistant and were used as markers for determination of a potential anti-TB efficacy of alkaloids.

**Sub-culturing of *Mycobacterium* species:** The strains were sub-cultured in Middlebrook 7H9 broth base supplemented with glycerol. The medium was prepared by suspending 1.18 g of Middlebrook 7H9 broth base in 230 mL of distilled water in a Scotch bottle (500 mL) followed by addition of 1 mL of glycerol (AR). The mixture was heated to dissolve the broth base completely, thereafter autoclaved at 121°C for 15 min. The mixture was left to cool to 31 and 35°C under lamina flow, before separately being inoculated with *Mycobacterium madagascariense* (MM) and *Mycobacterium indicus* Pranii (MIP), respectively. Thereafter, MM was incubated at 31°C while MIP was incubated at 37°C. The optimal growth of the bacteria cultures was observed after 5 days and thus ready for antimycobacterial screening.

**Determination of minimum inhibitory concentration (MIC):** The MIC values of alkaloids against two *Mycobacterium* strains were determined by two fold microdilution method as documented in literatures (Eloff, 1998; Erasto *et al.*, 2011).

**Potentiation of antimycobacterial activity of isoniazid using indole alkaloids:** Isoniazid (INH) has generally been found to be inactive against *M. madagascariense* (MM) and *M. indicus* pranii (MIP) even at higher concentration. This provided the opportunity to investigate the ability of indole alkaloids to potentiate the efficacy of INH against MM and MIP. Adopting the method of Eloff (1998) with modification, the Fractional Minimum Inhibitory Concentration (FMIC) of alkaloids was determined by screening 1/2 to 1/16 MIC values of alkaloids against MM and MIP, blended with 1/2 to 1/16 of the documented MIC value of isoniazid (INH) against *M. tuberculosis*. The MIC of INH was adopted from a previously recorded value against *Mycobacterium tuberculosis* (Mtb H37Rv) which is 8.75 µM (~0.12 µg mL<sup>-1</sup>) (Lawal *et al.*, 2011). This implied that, the first wells had 1/2 MIC values of alkaloid and INH which was then diluted two folds to the last well which had 1/16 MIC values of test samples. The controls in this assay were as follow; two rows with alkaloid (1/2 to 1/16 MIC values), mycobacteria innoculums and broth only, two rows with INH, mycobacteria innoculum and broth only and a positive control which had ciprofloxacin, mycobacteria innoculum and broth. The FMIC values of alkaloids was determined by addition of

40  $\mu\text{L}$  ( $0.2 \text{ mg mL}^{-1}$ ) iodonitrotetrazolium (INT) chloride salt into each well and plates incubated at 31 (MM) and 37°C (MIP) for 1 h. The FMIC values were read at the concentration where a marked no change in color formation as a result of INT metabolism by active mycobacteria was observed.

**Brine shrimp toxicity assay (BST):** The potential cytotoxicity effect of indole alkaloids was determined using a method described by Meyer *et al.* (1982).

**Data analysis:** The mean results of the percentage mortality from the brine shrimps lethality test were plotted against the logarithm of concentrations using the Fig. 1 computer program. Regression equation obtained from the graphs was used to obtain  $\text{LC}_{16}$ ,  $\text{LC}_{50}$  and  $\text{LC}_{84}$  and the 95% CI values. An  $\text{LC}_{50}$  value greater than  $100 \mu\text{g mL}^{-1}$  was considered to represent non-toxic compound (Nondo *et al.*, 2011).

## RESULTS

**Structure elucidation of indole alkaloids:** The phytochemical analyses of leaves and stem barks of *H. rubrostipulata* yielded two indole alkaloids namely mitraphylline (1) and isomitraphylline (2) (Fig. 1). Isomitraphylline was isolated as yellow solid, with a melting point (m.p)  $110^\circ\text{C}$ , while mitraphylline was obtained as white needle crystals with m.p.  $264^\circ\text{C}$ . The structures of the two compounds were established with the aid of 1-D and 2-D NMR analyses and by comparison with the existing spectral data in literatures (Saxton, 1965; Seki *et al.*, 1993; Pandey *et al.*, 2006). The important signals in the  $^1\text{H}$ -NMR spectrum ( $\text{CDCl}_3$ ) of isomitraphylline and mitraphylline were the doublets of the C-18 protons which appeared at  $\delta$  1.10 and 1.12 ppm respectively. Furthermore, the presence of prochiral protons resonating at  $\delta$  2.50 ppm (dd, 3, 6 Hz) and  $\delta$  3.39 ppm (dd, 6 Hz) ascribed to C-5 methylene protons and  $\delta$  2.46 ppm (dd, 2, 6 Hz),  $\delta$  2.03 ppm (dd, 6, 12 Hz) ascribed to C-6 as well as the isotropic protons at  $\delta$  2.36 ppm (bs) and  $\delta$  1.24 ppm (m) ascribed to C-14 confirmed the resemblance of the  $^1\text{H}$  NMR data of mitraphylline with those available in literature (Seki *et al.*, 1993). The  $^{13}\text{C}$  NMR spectra also showed important peaks at  $\delta$  167.1 and  $\delta$  181.3 ascribed to C-2 and C-22 carbonyl carbons of the mitraphylline and isomitraphylline skeletons, respectively. Since the two compounds are isomers, the observed stereochemical difference involve the chemical shifts of C-7, whereby in mitraphylline it has an R

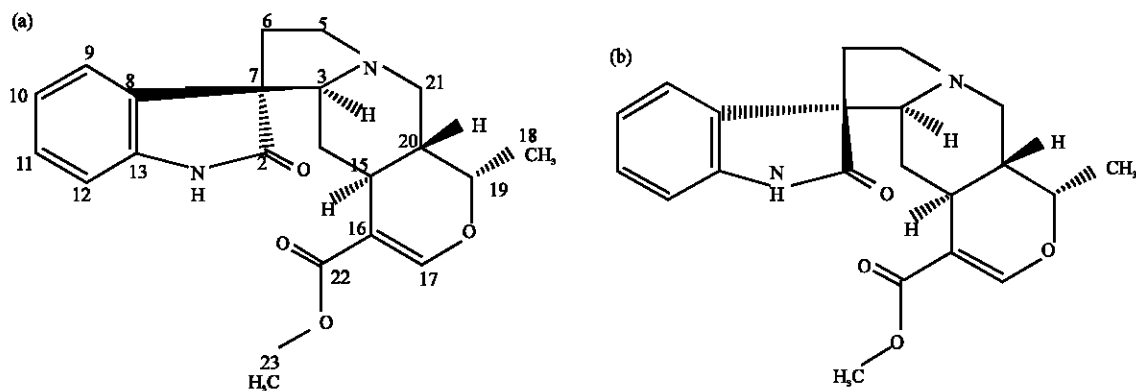


Fig. 1(a-b): Chemical structures of (a) Mitraphylline (1) and (b) Isomitraphylline (2) isolated from the leaves and stem bark of *Hallea rubrostipulata*

Table 1: Antimycobacterial activity of mitraphylline (1) and isomitraphylline (2)

Alkaloids and combination of alkaloids with isoniazid	Minimum inhibitory conc. (MIC) (mg mL <sup>-1</sup> )		Fractional minimum inhibitory conc (FMIC) (mg mL <sup>-1</sup> )	
	MM*	MIP**	MM	MIP
Mitraphylline 1	0.8	0.4		
1+INH <sup>a</sup>			0.4	0.2
1 (1/2 to 1/16 MIC values)			Na <sup>b</sup>	NA
Isomitraphylline 2	0.8	0.8		
2+INH			0.4	0.1
2 (1/2 to 1/16 MIC values)			NA	NA
Isoniazid (INH)	NA	NA		
Ciprofloxacin (µg mL <sup>-1</sup> )	0.05	0.05		

<sup>a</sup>Combination of alkaloid with isoniazid (INH), <sup>b</sup>No activity; MM\*: *Mycobacterium madagascariense*, MIP\*\*: *Mycobacterium indicus pranii*

configuration with a chemical shift of  $\delta$  55.6 ppm, while isomitraphylline has an S configuration C-7 resonating at  $\delta$  56.4 ppm. This corroborate with reported spectral data by previous researchers (Seki *et al.*, 1993; Pandey *et al.*, 2006).

Further observation of the <sup>13</sup>C NMR spectrum showed peaks at  $\delta$  71.8 and 74.0 ppm ascribed for C-3 and C-19 of isomitraphylline respectively, while in the <sup>13</sup>C NMR spectrum of mitraphylline these carbons resonated at 73.8 and 74.6 ppm, respectively. These differences confirmed further the stereochemical and thus structural differences of the two alkaloids. The C-5 and C-21, though sp<sup>3</sup> carbons, resonated at  $\delta$  54.3 ppm quite deshielded because of the electron withdrawing effect of the nitrogen atom. The literature show that the chemical shifts of these carbon atoms in the two compounds may sometime vary slightly although they are almost in the same chemical environment (Saxton, 1965). These data confirmed the identity of two indole alkaloids as mitraphylline (1) and isomitraphylline (2) which have also been previously reported from other *Hallea* species (Saxton, 1965; Seki *et al.*, 1993).

**Antimycobacterial activity of indole alkaloids:** The antimycobacterial screening of the two indole alkaloids revealed mitraphylline (1) to be more active than isomitraphylline (2). The former had MIC values of 0.8 and 0.4 mg mL<sup>-1</sup> against *M. madagascariense* (MM) and *M. indicus pranii* (MIP), respectively, while the latter had MIC values of 0.8 mg mL<sup>-1</sup> against both strains (Table 1). The two alkaloids are stereoisomers; hence observed slight difference in their antimycobacterial activity may be influenced by the configurational differences of chiral centres.

**Potential of antimycobacterial efficacy of isoniazid (INH):** The two mycobacteria strains have been reported to be resistant against INH even at higher concentration. This provided the opportunity to investigate whether indole alkaloids can potentiate the activity of INH against INH-insensitive *Mycobacterium* strains. The assay was done by blending ½ to 1/16 of MIC values of alkaloids with those of INH (used MIC value of INH against *M. tuberculosis*). The alkaloids-INH combinations exhibited appreciably higher activity, while individual alkaloids tested separately as controls (1/2 to 1/16 MICs of alkaloids and INH) lacked efficacy. Mitraphylline had the fractional MIC values of 0.4 and 0.2 mg mL<sup>-1</sup> while isomitraphylline had fractional MIC values of 0.4 and 0.1 mg mL<sup>-1</sup> against MM and MIP, respectively (Table 1). This implied that, the two indole alkaloids can potentiate the efficacy of INH against MM and MIP.

Table 2: Brine shrimp toxicity effects of mitraphylline (1) and isomitraphylline (2)

Compounds	LC <sub>50</sub> (µg mL <sup>-1</sup> )	95% Confidence interval (µg mL <sup>-1</sup> )
Mitraphylline (1)	3995.4	1201.7-12925.9
Isomitraphylline (2)	48.5	40.6-58.0
*Cyclophosphamide	16.3	10.6-25.2

\*Standard cytotoxic drug/anticancer drug

**Brine shrimp toxicity test:** Brine Shrimps Test (BST) is a general bioassay that helps to determine the potential cytotoxicity effect of compounds (Peteros and Uy, 2010). This assay also provides a front line screen that can be backed up by more specific and expensive bioassays. In this assay mitraphylline (1) was found to be nontoxic to shrimps with an LC<sub>50</sub> value of 3995.4 µg mL<sup>-1</sup> while, isomitraphylline (2) was slightly toxic to shrimps with an LC<sub>50</sub> value of 48.5 µg mL<sup>-1</sup> (Table 2). In this assay cyclophosphamide a standard anticancer drug (cytotoxic drug) had an LC<sub>50</sub> value of 16.3 µg mL<sup>-1</sup>. When compared with the standard drug, mitraphylline is non-toxic to shrimps compared to isomitraphylline. The cytotoxicity difference between the two indole alkaloids may be due to their stereochemistry at C-7, whereby in mitraphylline it has an R-configuration while in isomitraphylline it has an S-configuration.

## DISCUSSION

The phytochemical analysis on *Hallea rubrostipulata* has for many years attracted attention from scientist. This is due to its wide medicinal use, particularly on parasitic and microbial infections (Saxton, 1965; Shellard *et al.*, 1969; Taniguchi *et al.*, 1978; Seki *et al.*, 1993; Pandey *et al.*, 2006). The use of leaf extracts in the treatment of TB related infection as reported from Uganda (Taniguchi *et al.*, 1978) and revelation from ethnobotanical survey in Karagwe district raised the interest to conduct phytochemical isolation of major compounds which may be responsible for the claimed anti-TB efficacy. Of the isolated compounds, mitraphylline (1) was obtained in significant amounts compared to isomitraphylline (2). This implies that its abundance in *H. rubrostipulata*, together with the effect of other molecules hither-to unidentified, contribute immensely to the efficacy of this species in the treatment of TB related infections. Furthermore, the dichloromethane extract of the leaves of this plant species has recently been reported to exhibit higher antimycobacterial activity against MM and MIP (Chrian *et al.*, 2011). This shows that, the abundance of mitraphylline and its activity against MM and MIP correlate with the reported antimycobacterial activity of dichloromethane extracts of *H. rubrostipulata*. The structural differences of the two alkaloids especially the stereochemistry of carbon C-7 together with other stereogenic centers may be responsible for the observed disparities of antimycobacterial efficacy of the two indole alkaloids. This apparently favors mitraphylline (1) than its isomer, isomitraphylline (2) (Table 1).

Isoniazid has been reported to be inactive against *M. madagascariense* and *M. indicus pranii*, even at higher concentration (Kazda *et al.*, 1992; Saini *et al.*, 2009; Erasto *et al.*, 2011; Chrian *et al.*, 2011). This was again confirmed in this study where INH lacked efficacy against the two microbes. Unfortunately, there has been no report explaining the reason why the two mycobacteria strains are resistant to INH. Consequently, this provided an opportunity to investigate the efficacy of separate combinations of INH with mitraphylline and isomitraphylline against MM and MIP. The two alkaloids potentiated the activity of INH against the two insensitive mycobacteria strains. This implies that indole alkaloids circumvented the resistance mechanisms



of the two microbes and to enable INH to act at a much lower concentration than its MIC value against *M. tuberculosis* (Mtb). The mode of action of INH against Mtb is by inhibition of cell wall synthesis of the microbe. In this mechanism the primary target is a protein InhA. This is an enzyme responsible for the chain elongation of mycolic acid in the *Mycobacterium* cell wall.

The inhibition of InhA enzyme implies inhibition of cell wall synthesis in the *Mycobacterium* cell. Although it is not yet established, MM and MIP may possibly be having a different or a modified protein rather than InhA and thus a different sequence of a KatG gene. Another possible reason for resistance may be that MM and MIP lacks KatG gene, hence resembles the resistance of *M. tuberculosis* which is a result of mutation or loss of a KatG gene (Zhang *et al.*, 1992; Jaber *et al.*, 1996), this however needs further investigation. This study has therefore created an important aspect which needs further investigation. This includes studying the mode of action through which indole alkaloids activate INH at the range of  $\frac{1}{2}$  to  $\frac{1}{4}$  of their MIC values against resistant mycobacteria strains. A successful investigation of this knowledge gap shall pave way for use of MM and MIP strains as Mtb surrogates in the search for new antitubercular agents capable of breaking drug resistance in TB patients. The cytotoxicity assay revealed that mitraphylline and isomitraphylline are not toxic to shrimps as they had LC<sub>50</sub> values of 3995.4 and 48.5  $\mu\text{g mL}^{-1}$ , respectively, values higher than a standard drug which had an LC<sub>50</sub> value of 16.3  $\mu\text{g mL}^{-1}$ . This confirms why *H. rubrostipulata* has for many years been used traditionally in the treatment of diseases including respiratory tract infections.

## CONCLUSION

Alkaloids have generally been reported to have antimycobacterial efficacy. The two indole alkaloids investigated in this study exhibited higher antimycobacterial activity. Furthermore, the combination experiment revealed that indole alkaloids potentiate the efficacy of INH against INH-resistant *Mycobacterium* strains. Therefore mitraphylline (1) which is not toxic and has higher antimycobacterial activity both when tested alone and in combination with INH can be considered for further investigation, especially screening against MDR-TB strains. Work is in progress to determine the molecular basis of this biological effect which shall provide more information on the mode of action through which alkaloids circumvent INH resistance in the two mycobacteria strains.

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