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Antimycobacterial, antioxidant activity and toxicity of extracts from the roots of *Rauvolfia vomitoria* and *R. caffra*

Rauvolfia vomitoria ve *R. Caffra* kök ekstraktlarının antimikobakteriyel, antioksidan etkinliği ve toksisitesi

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SUMMARY

AIM: This study aimed to investigate the antimycobacterial activity of total alkaloid and ethanolic aqueous extracts of *Rauvolfia vomitoria* and *R. caffra*. It further aimed to evaluate antioxidant activity and cytotoxicity effect of the extracts which would have shown high antimycobacterial efficacy.
METHODS: The alkaloids from the roots of two plant species were extracted according to standard procedures. Antimycobacterial activity of the extracts was evaluated using a two fold microdilution method. The antioxidant activity of the alkaloids and ethanolic extracts was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and reducing capacity assays. The potential cytotoxicity of the extracts was evaluated using brine shrimp toxicity assay. Comparison of alkaloidal composition of the two alkaloidal extracts was done using HPLC analysis.
RESULTS: The study revealed that, alkaloid extracts from the roots of Rauvolfia species had higher antimycobacterial activity against *Mycobacterium madagascariense* (MM) and *M. indicus pranii* (MIP). The minimum inhibition concentration (MIC) values of alkaloid extracts ranged between 0.078 to 1.25 mg/ml, while the ethanolic extracts were generally moderately active against test organism. The alkaloids and ethanolic aqueous extracts of *R. vomitoria* exhibited high antioxidant activity. In the cytotoxicity assay, extracts did not exhibit any toxicity against brine shrimps.
CONCLUSION: The alkaloids from the roots of *R. vomitoria* have high antimycobacterial and antioxidant activity. The alkaloids ability to inhibit the growth of two *Mycobacterium* strains may be associated to its antioxidant activity. The cytotoxicity of the most active extract is negligible when compared to the standard anticancer drug, this may explain why *R. vomitoria* is highly used in the treatment of various infectious and physiological ailments.
Keywords: *Rauvolfia vomitoria*, *Rauvolfia caffra*, *Apocynaceae*, antimycobacterial, antioxidant, brine shrimp toxicity.

ÖZET
AMAÇ: Çalışmada *Rauvolfia vomitoria* ve *R. caffra* alkaloid ve etanolik sıvı ekstrelerinin antimikobakteriyel etkilerinin araştırılması ve ayrıca, yüksek antimikobakteriyel etki gösterecek olan ekstrelerin antioksidan ve sitotoksik etkilerinin değerlendirilmesi amaçlandı.
YÖNTEM: Alkoloidler, standart prosedürlere göre iki bitkinin köklerinden ekstrakte edildi. Ekstrelerin antimikobakteriyel etkileri, iki kat mikrodilüsyon metodu kullanılarak değerlendirildi. Alkaloidlerin ve etanol ekstrelerin antioksidan etkileri 1,1-difenil-2-pikrilhidrazil (DPPH) ve indirgeyen kapasite analizleri kullanılarak saptandı. Ekstrelerin potansiyel sitotoksisitesi, tuzlu su karidesi toksisite analizi kullanılarak değerlendirildi. İki alkoloidal ekstrenin alkoloidal bileşiminin karşılaştırılması, HPLC analizi kullanılarak yapıldı.
BULGULAR: Çalışmamız, *Rauvolfia* türlerinin köklerinden elde edilen alkaloid ekstrelerin, *Mycobacterium madagascariense* (MM) and *M. indicus pranii* (MIP)'ye karşı yüksek antimikobakteriyel etkiye sahip olduğunu göstermiştir. Alkoloid ekstrelerin minimum inhibitör konsantrasyonları (MIC), 0.078-1.25 mg/ml arasında değişirken, etanolik ekstreler, genellikle orta derecede bir etki gösterdi. *R. vomitoria* alkaloid ve etanolik sulu ekstreleri yüksek antioksidan etki sergiledi. Sitotoksisite analizlerinde, ekstreler toksisite sergilemediler.
SONUÇ: *R. vomitoria* alkaloidleri yüksek antimikobakteriyel ve antioksidan etkiye sahiptir. Alkaloidlerin mikobakterilerin büyümesini önlemeleri, antioksidan etkilerine bağlı olabilir. Standart antikanser ilaçla karşılaştırıldığında, en aktif ekstrenin sitotoksisitesi önemsizdir. Bu durum, çeşitli enfeksiyöz ve fizyolojik hastalıkların tedavisinde *R. vomitoria*'nın neden çok yoğun bir şekilde kullanıldığını izah edebilir.
Anahtar Kelimeler: *Rauvolfia vomitoria*, *Rauvolfia caffra*, *Apocynaceae*, antimikobakteriyel, antioksidan, tuzlu su karidesi toksisitesi

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INTRODUCTION

Rauvolfia species are important medicinal plant throughout sub-Saharan Africa. These are mainly trees which grows up to 8 m high, mostly in gallery forests where fallow periods are prolonged. *Rauvolfia* species are commonly used in the treatment of malaria, diabetes, and both parasitic and microbial infections [1-4]. Although different *Raulvofia* species such as *R. caffra* and *R. vomitoria* grow in Tanzania, *R. vomitoria* is the most used species in the treatment of various diseases. It is used in the treatment of coughs, gastrointestinal disturbances, skin infections, hypertension, impotence, insomnia, diarrhea, dysentery, scabies, worm infections and malaria [5,6]. These ethnomedical uses make it one of the most important medicinal plants used in the suppression of skin diseases and opportunistic infections in HIV/AIDS patients in Tanzania (unpublished data).

Rauvolfia vomitoria Afzel. and *R. caffra* Sond. (Apocynaceae) are rich in indole alkaloids most of which have been isolated and identified [7,8]. The phytochemical analysis on *R. vomitoria* revealed roots to have high concentration of reserpine and ajmaline. These alkaloids have various pharmacological properties including antimalarial, anti-tumor and antidiabetes efficacy [9,10]. Plants species which contain compounds with strong conjugation system such as reserpine and ajmaline have high antioxidant activity therefore capable of reducing the risks of certain type of tumors and other degenerative diseases [11,12]. Furthermore, the extracts and alkaloids derived thereof may have high antimicrobial activity which is due to inhibition of some redox pathways and other biochemical processes in the bacterium cell. As part of our efforts to search for extracts and compounds with high antimycobacterial efficacy, the roots extracts of *R. vomitoria* and *R. caffra* were screened for antimycobacterial activity. The species with the most active extract was screened for antioxidant and cytotoxicity activity so as to evaluate further pharmacological effects and safety for its use in ethnomedicine.

MATERIALS AND METHODS

Chemicals

Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Potassium ferricyanide [$K_3Fe(CN)_6$], Trichloroacetic acid, Ferric chloride and cyclophosphamide were purchased from Sigma Aldrich (South Africa). 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), iodonitrotetrazolium (INT) chloride, Ciprofloxacin (R&D) and Isoniazid (R&D) were purchased from Sigma (UK), 96 wells microtitre plates supplied by KAS Medics (Tanzania).

Test organisms

The two test organisms namely *Mycobacterium madagascariense* (MM) DSM 44641 and *Mycobacterium indicus pranii* (MIP) DSM 45239 were supplied by DSMZ - the Germany Resource Centre for Biological Materials, Braunschweig, Germany. The two mycobacteria strains were used as markers for determination of a potential anti-TB efficacy of various extracts.

Collection and extraction of plant materials:

The roots of *Rauvolfia vomitoria* were collected from Bukoba while *R. caffra* was collected from Lushoto Tanga, Tanzania. The plants were identified by Mr. E.B. Mhoro (retired botanist) from the Botany department of the University of Dar es Salaam, Tanzania. The voucher specimen Mh 11464 and Mh 11463 are deposited in the herbarium at the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences. The roots were chopped into small pieces and air dried under shade before pulverization. 250 g of the ground materials of each plant species were shaken in 80% ethanolic aqueous for 24 hrs. The extracting solvent was used with reference to folk drug preparation where they use alcohol and water to extracts the plant materials. The extracts were concentrated under reduced pressure at 50^{o}C to give 15.2 g and 9.7 g of *C. vomitoria* and *C. caffra* respectively. The portion of the extracts was partitioned for total alkaloid extraction, while the remaining ethanolic aqueous extract was kept for biological analyses.

Total alkaloid extraction

A modified method of Rujjanawate et al. [13] was used to extract the alkaloids from the root extracts of *R. vomitoria*. 10.46 g of the extract was dissolved in 300 ml distilled water in a 500 ml beaker. The beaker and its content were shaken in the water bath set at 40^{o}C to further dissolve the extract and obtain a homogenous solution. Thereafter the crude mixture was acidified by adding 10 ml of 5% sulphuric acid in water. Subsequently, the acidic solution was repeatedly washed with 100 ml CH_2Cl_2 to remove neutral substances. The aqueous acidic solution was then made basic with 15

ml of 20% NH_4OH and extracted again with CH_2Cl_2 until the aqueous layer was free of alkaloids. The combined total CH_2Cl_2 extracts were evaporated in vacuo to yield 2.41 g of the alkaloid as a shiny brown powder. The crude alkaloids were spotted on a thin layer chromatographic plate and developed using dichloromethane/methanol (10:1). In order to confirm the presence of alkaloids, the TLC plate was sprayed with Dragendorf reagent to give three major orange spots and some several other minor compounds. Similar method was used in the extraction of alkaloids from the roots of *C. caffra*.

Sub-culturing of Mycobacterium species

The *Mycobacterium* strains were sub-cultured in Middlebrook 7H9 broth base supplemented with glycerol. 1.18 g of Middlebrook 7H9 broth base was suspended 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^{o}C for 15 minutes. The mixture was left to cool to 31 and 35^{o}C under lamina flow, before separately being inoculated with MM and MIP respectively. Thereafter MM was incubated at 31^{o}C while MIP was incubated at 37^{o}C. The optimal growth of the bacteria cultures was observed after 5 days, and thus ready for antimycobacterial screening.

Determination of minimum inhibition concentration (MIC) of extracts

The MIC values of extracts against two *Mycobacterium* marker strains was determined by two fold microdilution method [14], in the sterile flat - bottomed 96 - well polystyrene microtiter plates. Bacterial inoculums were prepared from five days grown cultures in middlebrook 7H9 broth base containing 0.1% tween 80 and the turbidity was adjusted to the equivalent of 0.5 McFarland units (approximately 1.2 x10^8 CFU/ml). The concentration of stock solution of all test extracts before serial dilutions was 20 mg/ml. The extracts were serially diluted two folds with a broth base containing 0.1% tween 80. The serial dilution was performed by addition of 50 µl of extracts into the first well which had 50 µl of broth base, and thereafter mixed well and transferred 50 µl of the first well sample-broth base mixture to next and subsequent wells of each row. The remaining 50 µl of the mixture was discarded from the last well of the row. This was followed by the separate inoculation of 50 µl of mycobacteria cultures in each well, to complete a two fold broth microdilution. Two additional wells were used as growth controls, where no drugs were added as negative control, and while a row with inoculums and control drugs were used as positive control. The inoculated microtiter plates were incubated at 31^{o}C for MM and 37^{o}C for MIP for 24 hours.

To determine the MIC values of extracts, 40µl (0.2 mg/ml) iodonitrotetrazolium (INT) chloride salt was added into each well and plates incubated at 31 and 37^{o}C for 1 hour. The minimal inhibitory concentration (MIC) value of each extract was read at the concentration where a marked reduction in color formation due to bacterial growth inhibition was noted. Isoniazid and Ciprofloxacin were used as positive controls.

Determination of DPPH radical scavenging activity

The method described by Liyana-Pathirana and Shahidi [15] was used to assess the DPPH radical scavenging activity of R. vomitoria root extracts. An amount of 0.5 ml of 0.12 mM DPPH solution in methanol was separately mixed with 2 ml of 0.01, 0.025, 0.05 and 0.075 mg/ml of the extracts/alkaloids in methanol and vortexed thoroughly. The absorbance of the mixture at ambient temperature was recorded for 30 minutes at 10 minutes intervals. Gallic acid (GA) was used as a reference antioxidant compound. The absorbance of the remaining DPPH radicals was read at 519 nm using a Jenway 6505 UV/Vis spectrophotometer (Essex, UK). The analysis of each assay solution was replicated thrice. The scavenging of DPPH radical was calculated according to the following equation:

DPPH radical scavenging activity (%) = [(Acontrol-Asample)/(Acontrol)] x 100

Where Acontrol is the absorbance of DPPH radical in methanol, Asample is the absorbance of DPPH radical + sample extract/standard.

Reducing capacity

Adopting the method of Oyaizu [16], 0.01, 0.025, 0.05 and 0.075 mg/ml of the root extracts were mixed with 2.5 ml of 0.02 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was then incubated at 50^{o}C for 20 min. Aliquots (2.5 ml) of 10% trichloroacetic acid were added to the mixture, which was then centrifuged for 10 min at 1000 x g. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$, and the absorbance was measured at 700 nm in a Jenway 6505 UV/Vis spectrophotometer. Gallic acid was used as a standard antioxidant compound.

The analysis of each assay solution was done three-times.

Brine shrimp toxicity test

Adopting the method of Meyer et al. [17] artificial sea water was prepared by dissolving previously generated sea salt in distilled water (38 g/l) and poured into a 23 x 3 cm glass tank divided into two unequal compartments by a perforated polystyrene wall. Brine shrimp eggs (0.5 g) were sprinkled into the larger compartment which was kept dark by covering on the sides and at the top. The smaller compartment was kept illuminated with an electric bulb. After 48 h the hatched, mature phototropic nauplii, were collected from the illuminated compartment using a micropipette. The stock solutions of extracts were prepared in dimethyl sulfoxide (DMSO) at concentrations of 24, 40, 80, 120 and 240 µg/ml ready for testing. Triplicate aliquots (2 ml) of each of the solution were added into vials and later made up to 10 ml with sea water. Ten brine shrimp larvae were transferred into each of the triplicate vials. The vials were maintained under illumination. Controls were placed in a mixture containing seawater and DMSO only. After 24 h the nauplii were examined against a lighted background and the average number of survived larvae in each triplicate was determined. Cyclophosphamide was used in assay as a standard anticancer drug.

Data analysis

The statistical analysis for the DPPH radical scavenging activity and reducing capacity was achieved using Excel. For the Brine shrimp lethality test, the mean results of the percentage mortality were plotted against the logarithm of concentrations using the Fig P computer program. Regression equation obtained from the graphs was used to obtain LC16, LC50 and LC84 and the 95% CI values. An LC50 value greater than 100 µg/ml is considered to represent inactive extract.

HPLC analytical conditions

The HPLC analysis of alkaloid extracts was carried on a Shimadzu LC-20AD HPLC system with an SPD-20A detector and STR ODS-II (150 mm, 4.6 mm I.D, 5µm) column. The mobile phase consisted of A methanol 80% and B 20% acetonitrile. The flow rate was 1.0 ml/min, and the detector was set at 254 nm to record the chromatograms. The column temperature was kept at 30 ± 0.15^{o}C, the injection volume was 5 µl and the run time was set at 17 minutes.

RESULTS

Antimycobacterial activity

The antimycobacterial efficacy of ethanolic and alkaloid extracts from the roots of *R. vomitoria* and *R. caffra* was determined by screening extracts against two non pathogenic *Mycobacterium* species namely; *Mycobacterium madagascariense* (MM) and *M. indicus pranii* (MIP). In this assay, the alkaloidal extracts (RV1) of *R. vomitoria* exhibited higher antimicobacterial activity against all test organisms. The extract had MIC values of 0.156 and 0.078 µg/ml against *M. madagascariense* and *M. indicus pranii* respectively (Table 1). The ethanolic aqueous extracts (RV2) had weak activity with MIC values of 2.5 µg/ml against all strains. The alkaloid extract (RC1) from the roots of *R. caffra* had MIC value of 1.25 µg/ml against all test organisms, ranking second from the alkaloids (RV1) of *R. vomitoria*. Comparing the activities of extracts and the standard antibiotics, Ciprofloxacin had much higher activities than all plant extracts (Table 1). However the Isoniazid which is one of the drugs used in the treatment of tuberculosis did not show any efficacy against the two *Mycobacterium* species.

Table 1: Antimicobacterial activity of the alkoloids and ethanolic extracts of *Raulvolfia Vomitoria* and *R. caffra*

Extract	Minimum Inhibition Concentration (MIC) in mg/ml	
	Mycobacterium madagascariense	***M. indicus pranii***
RV1	0.156	0.078
RV2	2.5	2.5
RC1	1.25	1.25
RC2	2.5	2.5
Ciprofloxacin	<0.05	<0.05
Isoniazid	NA	NA

[a]RV1: *R. Vomitoria* alkoloidal extract
RV2: *R. Vomitoria* ethanolic extract
RC1: *R. caffra* alkoloidal extract
RC2: *R. caffra* ethanolic extract

Table 2: Cytotoxicity activity (BST) of alkoloids and ethanolic extract of *Raulvolfia vomitoria*

Extract[a]	LC50 (µg/ml)	95% Confidence Interval (µg/ml)
RV1	81.6	59.54 – 111.04
RV2	47.9	34.07 – 67.36
Cyclophosphamide	16.3	10.60 – 25.15

[a]RV1: *R. Vomitoria* alkoloidal extract
RV2: *R. Vomitoria* ethanolic extract

Antioxidant activity

Since the extracts of the roots of *R. vomitoria* exhibited higher antimycobacterial activity than those of *R. caffra*, these were then subjected to antioxidant assay using free radical scavenging activity and reducing capacity assay. This was with the aim of consolidating further the observed antimycobacterial efficacy of the extracts.

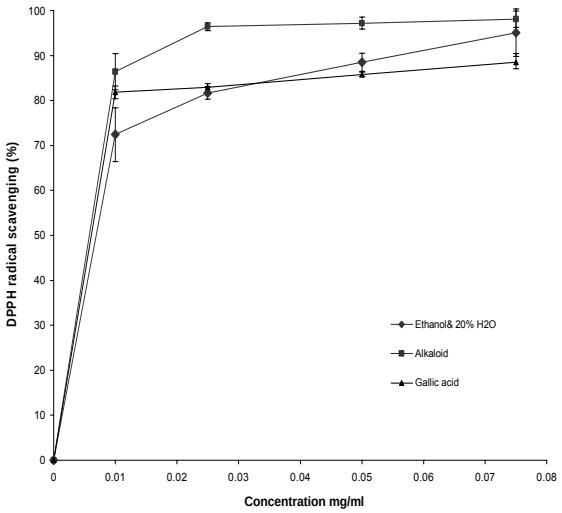

Fig. 1: The DPPH radical scavenging activity of ethanolic aqueous and alkoloid extract from the whole root of *R. vomitoria* compared with gallic acid after 60 min of reaction. Each value is expressed as mean±SD (n=3)

DPPH radical scavenging activity

The free radical scavenging activity of the 80% ethanolic aqueous extract (RV2) and alkaloids (RV1) from the root of *Rauvolfia vomitoria* was determined from a reduction of absorbencies of DPPH radical at 519 nm. In this assay, both 80% ethanolic aqueous and alkaloid extracts exhibited high antioxidant activity than gallic acid, a standard and natural antioxidant compound (Fig. 1). The order of activity was alkaloids (RV1) > 80% ethanolic aqueous (RV2) > Gallic acid. At 0.05 mg/ml the alkaloid extract scavenged 97.2 % of the DPPH radical while 80% ethanolic aqueous extract had 88.5% and gallic acid had 85.8% (Fig. 1). It was further observed that the activity of each test sample increased with concentration and time.

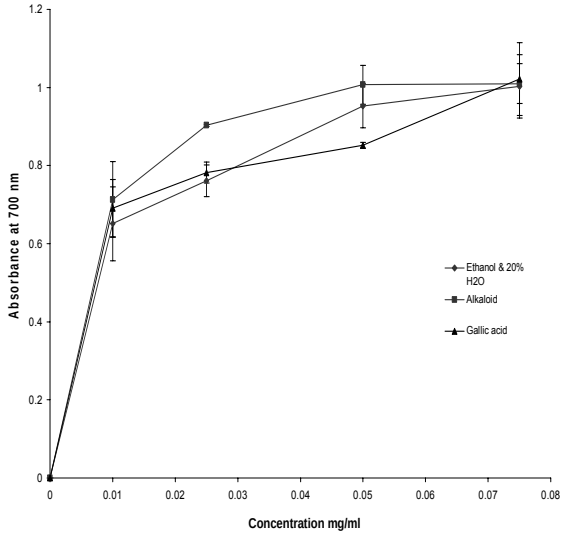

Fig. 2: Reducing capacity of different amounts of ethanolic aqueous and alkoloid extract from the whole root of *R. vomitoria* compared to gallic acid (a standart antioxidant compound) using spectrophotometric detection of $Fe^{3+} \rightarrow Fe^{2+}$ transformation. Each value is expressed as mean±SD (n=3)

Reducing capacity

The antioxidant activity of the roots of *R. vomitoria* was further manifested through their reducing power as shown in figure 2. In this assay, the $Fe^{3+} \rightarrow Fe^{2+}$ transformation was established as reducing capacity. Again alkaloids (RV1) had superior reducing power than the other assayed samples, followed by 80% ethanolic aqueous (RV2) extract and then gallic acid. At 0.05 mg/ml the absorbencies of alkaloids, 80% ethanolic aqueous

extract and gallic acid (at 700 nm) were 1.0, 0.953 and 0.852 respectively. However, at 0.075 mg/ml the absorbencies of all samples were of the same order (Fig. 2). This trend shows that reducing capacity increased with increasing concentration of the sample, thus implying that the strength of donation of electrons is directly related to the concentration of the extracts.

Brine shrimps lethality test

The brine shrimp lethality test (BST) was used to predict cytotoxicity properties of both 80% ethanolic aqueous extract and the alkaloid extracts of R. vomitoria. Results revealed that all extracts were less toxic to brine shrimps (Table 2). Ethanolic aqueous extract had LC50 value of 47.9 µg/ml while the alkaloids had LC50 value of 81.6 µg/ml (Table 2). All test samples were far less toxic to shrimps compared to a standard anticancer drug cyclophosphamide which had LC50 value of 16.3 µg/ml.

HPLC Profiles of the alkaloid extracts of R. vomitoria and R. caffra

The HPLC profiles of the alkaloid extracts of the two plant species was developed so as study the similarities and differences in terms of number of alkaloids present and their abundances. As shown in figure 3 the alkaloid constituents of the two *Rauvolfia* species vary significantly. The alkaloid extract (RV1) from the roots of *R. vomitoria* showed one major peak at RT 2.45 minutes, with the rest being minor compounds (Fig. 3A). This contrast significantly with the profile of the alkaloids extracts (RC1) from the roots of *R. caffra*, which showed a major peak at RT 2.73 min, and several other compounds at RT 2.43, 3.13, 3.70, 3.85, 4.04 and 6.24 minutes (Fig. 3B). However, the similarity between the two plant species is that, the major alkaloid in roots of *R. vomitoria* (RT 2.45 min), appeared to be minor in *R. caffra* (RT 2.43 min). This similarity, and the differences in term of number of compounds and their abundances, may explain the variation of biological activities of the alkaloid extracts of the two species.

CONCLUSION

The ethanolic aqueous and alkaloid extracts of the roots of *R. vomitoria* and *R. caffra*, exhibited moderate to high antimycobacterial activity against MM and MIP. The alkaloid extracts of the roots of *R. vomitoria* were more active than all test samples with MIC values of 0.156 and 0.078 mg/ml against MM and MIP respectively. The HPLC profile of the alkaloid extracts of the two species revealed a significant difference in terms of number of compounds present in the extract and their abundances. For instance in *R. vomitoria*, the alkaloid extract showed only one major peak, with the rest being minor constituents. The influence of one major alkaloid is evident in the MIC values of *R. vomitoria* extract as compared to the MIC values of alkaloid extract of *R. caffra*. The alkaloid with RT 2.43 min appears to be the minor component in *R. caffra*, and this may explain why the activity of *R. vomitoria* alkaloid extract is higher than that of *R. caffra.* The two organisms were all not sensitive to isoniazid a standard anti-TB drug used as a positive control. This observation agrees with reported studies on both *Mycobacterium* species [18, 19].

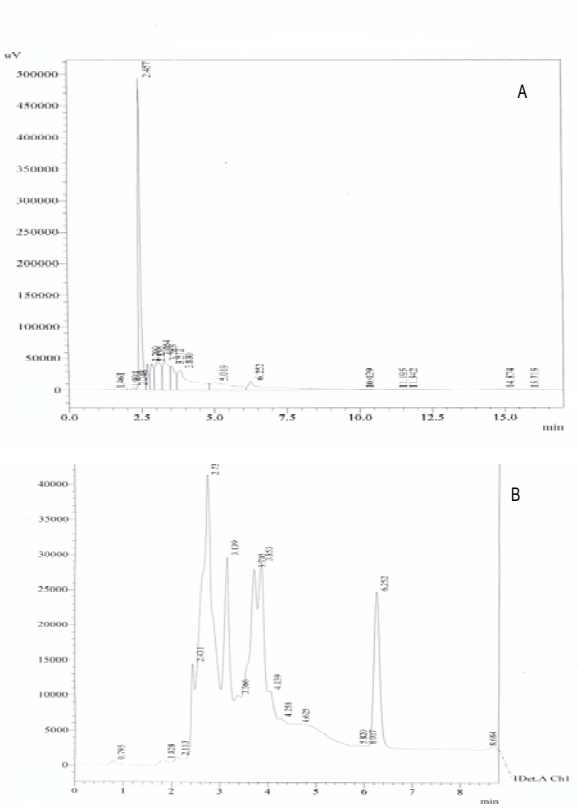

Fig. 3: HPLC chemical fingerprints of alkoloid extracts from the roots of *Rauvolfia vomitaria* (A) and *Rauvolfia caffra* (B).

The ethanolic aqueous extracts of both species exhibited the same antimycobacterial activity with MIC values of 2.5 mg/ml. When this is compared to the MIC values of alkaloids, it simply explains that,

there exist some compounds in the aqueous extracts which have antagonistic effects to alkaloids. The difference in activity of alkaloids and ethanolic aqueous extract is appreciably high, this makes alkaloids of this species be the compounds of interest for further anti-TB studies.

The root extracts of *R. vomitoria* were further subjected on antioxidant and cytotoxicity studies. This is because the alkaloids of *R. vomitoria* exhibited higher antimycobacterial efficacy, hence the need to explore further pharmacological effects and safety. The alkaloid extracts exhibited higher free radical scavenging activity than gallic acid and ethanolic aqueous extract of the roots of *R. vomitoria.* It further exhibited good electron donating ability, which implies that alkaloids may be inhibiting some redox pathways in the *Mycobacterium* cell, thereby slow the growth or even cause death of a microbe. This pharmacological property adds value to the potential antimycobacterial efficacy of the alkaloids from the roots of *R. vomitoria.*

The electron donating ability of the alkaloid is very important in the inhibition of the *Mycobacterium* cell growth. This is because a Mycobacteria cell, utilizes NADPH dependent mycothione reductase enzyme to maintain an intracellular reduced environment in the cells. The enzyme reduces a symmetrical mycothiol disulfide (MSSM) into thiol mycothiol (MSH). This is a continuous cycle which helps to protect the mycobacteria cell from oxidative stress [20]. The alkaloids in the roots of *R. vomitoria*, may have better binding site for the mycothione reductase, hence becomes subversive substrates in the thio-redox pathway of a *Mycobacterium* cell. This important biochemical process needs to be studied further so as to confirm one of the possible modes of action of the alkaloids.

The cytotoxicity assay of alkaloids and ethanolic aqueous extract revealed both to be non toxic to shrimps. The alkaloids had LC50 value of 81.6 µg/ml far higher than that of the standard anticancer drug cyclophosphamide which had LC50 value of 16.3 µg/ml. The lower the LC50 value the higher the toxicity. Therefore, alkaloids from the roots of R. vomitoria are non-toxic, and this explains why people in the rural areas of Tanzania and elsewhere in Africa continue to use this plant species in the treatment of various infectious and physiological diseases.

The observed antimycobacterial and antioxidant efficacy of the alkaloids from the roots of R. vomitoria and the fact that an HPLC profile indicated one major compound necessitates further studies to identify the exact alkaloid responsible for this activity. Work is in progress to purify the major alkaloid and other minor compounds from the roots of this species. Thereafter screen the alkaloids for antimycobacterial activity and their ability to inhibit mycothione reductase redox pathway in the mycobacteria cell.

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