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Amos Marume
Department of Paraclinical Veterinary Studies, Faculty of Veterinary Science, University of Zimbabwe, MP167 Mt Pleasant, Harare, Zimbabwe

Gift Matope
Department of Paraclinical Veterinary Studies, Faculty of Veterinary Science, University of Zimbabwe, MP167 Mt Pleasant, Harare, Zimbabwe

Star Khoza
Department of Clinical Pharmacology, College of Health Sciences, University of Zimbabwe, P.O. Box A178, Avondale, Harare, Zimbabwe

Isaac Mutingwende
School of Pharmacy, College of Health Sciences, University of Zimbabwe, MP167, Mt Pleasant, Harare, Zimbabwe

Takafira Mduluza
Department of Biochemistry, Faculty of Sciences, University of Zimbabwe, MP167 Mt Pleasant, Harare, Zimbabwe

Tariro Mawoza
Department of Clinical Pharmacology, College of Health Sciences, University of Zimbabwe, P.O. Box A178, Avondale, Harare, Zimbabwe

Tariro D Chawana
Department of Clinical Pharmacology, College of Health Sciences, University of Zimbabwe, P.O. Box A178, Avondale, Harare, Zimbabwe

Tafadzwa Taderera
Department of Physiology, College of Health Sciences, University of Zimbabwe, MP167, Mt Pleasant, Harare, Zimbabwe

Ashwell R Ndhlala
Agricultural Research Council, Vegetable and Ornamental Plants (VOP), Private bag X923, Pretoria 0001, South Africa

Correspondence
Amos Marume
Department of Paraclinical Veterinary Studies, Faculty of Veterinary Science, University of Zimbabwe, MP167 Mt Pleasant, Harare, Zimbabwe

Antimicrobial and *in vitro* enzyme inhibitory activities of selected Zimbabwean ethno-veterinary medicinal plants used in folklore animal wound management

Amos Marume, Gift Matope, Star Khoza, Isaac Mutingwende, Takafira Mduluza, Tariro Mawoza, Tariro D Chawana, Tafadzwa Taderera and Ashwell R Ndhlala

Abstract
Plants are often justifiably used in folklore medicine. A determination of the antibacterial, antifungal and anti-enzymatic activities of the extracts of *Cissus quadrangularis*, *Adenium multiflorum* and *Erythrina abyssinica*. Microdilution bioassays were used for both antibacterial and antifungal activities. Bacteria used are *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Brevibacillus agri* and *Staphylococcus epidermis*. Fungi used are *Candida albicans*, *Trichophyton mentagrophytes*, *Trichophyton tonsurans* and *Microsporum canis*. Non-radioactive HIV-RT colorimetric ELISA was used for the anti-reverse transcriptase activities. *E. abyssinica* leaf and *A. multiflorum* crude extract fractions exhibited the best antibacterial and antifungal properties. Ethanol extract fractions gave the best antimicrobial properties. All plants exhibited good anti-reverse transcriptase-1 activities. *Erythrina abyssinica* leaf extracts gave the best anti-tyrosinase activity followed by *E. abyssinica* bark then *A. multiflorum*. Crude extracts of *E. abyssinica* leaf and bark gave the best anti-α-glucosidase activities and *C. quadrangularis* leaf also gave good activity.

Keywords: Antibacterial, antifungal, anti-reverse transcriptase, microdilution, anti-α-glucosidase, anti-tyrosinase

1. Introduction
Potential efficacy and safety of many herbal remedies and/or extracts thereof have been validated by both science and time. Many herbs or their extracts have been demonstrated to have significant antioxidant, antibacterial, antifungal, anti-inflammatory, antiviral, anticancer among other key biological effects. Most human and/or animal conditions like wounds are complicated by infections and complex inflammatory processes. Many of the infections are increasingly becoming resistant to conventional antimicrobial drugs through various mechanisms e.g. producing inactivating enzymes, limiting intracellular accumulation (active extrusion and/or restricted entry), altering target sites, alternative metabolic pathways and biofilm formation [1]. Complications resulting from "newer" diseases/conditions or phenomena like HIV/AIDS, hyper-virulence, cancers and/or malnutrition are further reducing the effectiveness of modern methods of disease management. This is exerting more pressure on scientists, policy makers and pharmaceutical companies to always be innovating novel methods of disease management [2, 3, 4]. Ethnoherbal medicinal plants or their extracts may provide the much needed option for the future. Phytoconstituents are often afforded their various biological effects by the complex mix of different molecules in herbal medicines with wide capabilities that include interfering with key enzymes in metabolic systems of humans and animals. An example could be the anti-tyrosinase, anti-lipase and anticholinesterases activities of bee pollen extracts, which explain their ability to prevent melanin associated cell death, inflammatory linked destruction of the pancreas and the etiopathogenicity of Alzheimer's disease, respectively [5]. Polyphenols or phenolics (e.g. flavonoids and stilbenes) are some of the most abundant phytoconstituents with an array of biological/pharmacological effects including antioxidant, anti-inflammatory, analgesic, antimicrobial and antipyretic properties [6]. All these effects are often key in determining the prognosis of several human and animal disorders. Wounds are common in both humans and animals and are often complicated with infection and chronic inflammation. Thus any plant with phytoconstituents that are able to address either or both of these possible complications will often have wound healing properties. A broader approach in the bioassays screening for possible pharmacological effects of herbal extracts is justified by the fact that plants have concoctions of phytochemicals with wide ranging effects.

Therefore, the objective of this study was to determine the antibacterial,antifungal, anti-HIV-1 reverse transcriptase, anti-tyrosinase, anti-α-glucosidase and anti-phospholipase A_2activities of crude extracts from *C. quadrangularis* (stems and leaves), *A. multiflorum* (whole aerial plant parts) and *E. abyssinica* (leaves and bark). Their uses include management of animal wounds, warts, eye infections and fractured bones (as well as bone dislocations) [7].

2. Materials and Methods

2.1 Plant collection and identification

The plant materials were collected from Mberengwa, Midlands Province (*Cissus quadrangularis* L. (Vitaceae)-20°28′09.0″S 29°55′23.3″E.), Karoi, Mashonaland West Province (*Erythrina abyssinica* Lam. Ex DC. (Fabaceae)-16°49′44.1″S 29°41′19.8″E) and Buhera, Manicaland Province (*Adenium multiflorum* Klotzsch (Apocynaceae)-19°17′10.7″S 31°25′20.2″E) of Zimbabwe during the months of October-December 2016. Species identification was done by qualified botanists from the National Herbarium and Botanic Garden, Harare and University of Zimbabwe where voucher specimens were deposited. Detailed plant use in ethnoveterinary medicine are listed in Marume *et al*. (2017).

2.2 Extraction and ointment preparation

Fresh whole aerial plant parts samples from *C. quadrangularis*, whole aerial plant parts of *A. multiflorum*, leaf and bark samples of *E. abyssinica* were separately oven dried at 50 °C for 48 h. Dried plant materials were ground into powders and extracted (1:20 w/v) with 50% aqueous methanol in an ultrasonic bath for one hour. The extracts were filtered under vacuum through Whatman's No. 1 filter paper. The extracts were then concentrated under pressure using a rotary evaporator at 30 °C and freeze dried overnight.

2.3 Solvent partitioning of the crude extract

For antibacterial and antifungal assays, the concentrated freeze dried crude extract was sequentially extracted with petroleum ether (PE), dichloromethane (DCM), 70% v/v ethanol and water. The solvent fractions were then concentrated to dryness *in vacuo*at 30 ºC.

2.4 Antimicrobial bioassays

2.4.1 Antibacterial microdilution bioassay

The six bacteria chosen: *Bacillus subtilis* ATCC 6051, *Staphylococcus aureus* ATCC 12600, *Escherichia coli* ATCC 11775, *Klebsiella pneumoniae* ATCC 13883, *Brevibacillus agri* and *Staphylococcus epidermidis* included those that can be pathogenic to human and animal skins, wounds or other body tissues. The antibacterial properties were assayed as detailed by Ndhlala *et al*. (2011), Aderogba *et al*. (2013) and Madikizela *et al*. (2017) with minor modifications. The microdilution method using 96-well microtiter plates (Greiner Bio-one GmbH, Frickenhausen, Germany) was used to determine the antibacterial properties i.e. the minimum inhibitory concentration (MIC) values of the fractions of the crude methanolic extracts [8, 9, 10] One hundred microlitres of the re-suspended crude extract fractions (50 mg/mL) were two-fold serially diluted with purified and/or sterile distilled water in duplicate down the microtiter plate for each of the six bacteria used. Similar dilutions of neomycin (0.1 mg/mL) was used as a positive control against each bacterium. The screening was repeated twice for each extract.

2.4.2 Antifungal micro dilution bioassay

The screening was done using all the crude extract fractions as in antibacterial bioassay. Four fungal species: *Candida albicans* ATCC 10231, *Trichophyton mentagrophytes*, *Trichophyton tonsurans* and *Microsporum canis*) also known to inhabit or infect human and animal skins, wounds and other deep tissues were also chosen for antifungal screening of the crude extract fractions. The screening of the extract fractions was done as in antibacterial screening in 4.2.4.1. Using micro dilution assays modified for fungal assays [8, 9]. The screening was also done in duplicates and in this case a similar 2-fold serial dilution of amphotericin B (2.5 mg/mL) was used as the positive control for every fungi used. Minimum inhibitory concentrations (MICs) values for each extract fractions were recorded.

2.5 Enzyme Inhibition Bioassays

2.5.1 HIV-1 reverse transcriptase inhibitor bioassay

The effect of the crude methanolic extracts and the water fractions on reverse transcription were evaluated under nuclease-free conditions following protocols supplied with the kits used (i.e. non-radioactive HIV-RT colorimetric enzyme-linked-immunosorbent serologic assay (ELISA) kit, Roche Diagnostics, Germany) as detailed by Ndhlala *et al*. (2010). Detection and quantification of synthesized DNA measures the activity of the reverse transcriptase enzyme which is followed by the sandwich ELISA protocol i.e. fresh Biotin-labelled DNA, binds to the surface of microtiter plate modules with wells that are pre-coated with streptavidin [11]. The labelled DNA then binds to an antibody conjugated to peroxidase (anti-DIG-POD) in the next step. In the final step, a coloured reaction product of the action of the peroxidase on added 2, 2'-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) is measured spectrophotometrically. Three tubes containing water instead of sample extracts were used as negative controls. Lamivudine (1.0 mg/mL)/zidovudine (2.0 mg/mL) combination (Combivir®) from GlaxoSmithKline and lopinavir (8.9 mg/mL)/ritonavir (2.2 mg/mL) combination (Kaletra®) from Abbott were used as positive controls. The inhibitor concentrations (IC_{50}) reducing RT activity by half of herbal preparations were then calculated using GraphPad Prism (version 4.0-GraphPad Software Inc.) statistical software programme for Windows and results presented as means with± standard errors [11]. The experiments were done in duplicates for each extract.

2.5.2 Tyrosinase inhibitory activity

Tyrosinase, a multifunctional copper-containing enzyme with key roles in the melanisation human skin, is also widely distributed in other living organisms like fungi, plants and animals. The enzyme inactivation is key in perceived beauty through discolouration (i.e. less dark pigmentation). A modified 96 well microtiter plate bioassay was used to measure tyrosinase inhibition through measuring absorbance at 492 nm. The crude methanol plant extracts were combined with 30 µL of the enzyme tyrosinase (333 units/mL in potassium phosphate buffer at pH 6.5). To the incubated extract-enzyme mixture 110 µL of 12 mM L-DOPA was added to each well. The negative control was all the compounds except for the substrate (L-DOPA) and kojic acid was used as the positive control [12, 13, 14]. After further 30 minutes incubation at room temperature, the percentage inhibition of the tyrosinase enzyme (IC_{50}) was calculated based on equation 1.

2.5.3 α-Glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity of the methanol crude extracts was assayed as detailed by Aderogba *et al*. (2013) and Madikizela *et al*. (2017) with relevant modifications. In the preparation of the enzyme solution, yeast α-glucosidase (0.1 Unit/mL) was dissolved in 0.1 M potassium phosphate buffer at pH 6.8. The same buffer was used to prepare the substrate i.e. 0.375 mM p-nitrophenyl-α-D-glucopyranoside (pNPG). After mixing the sample (20 µl), enzyme solution (20 µl) and substrate (40 µl), incubation for 40 minutes at 37 ^{o}C and reaction stopping (with 80 µl of 0.2M sodium carbonate in 0.1M potassium phosphate buffer at pH 6.8); the product p-nitrophenol (pNP) released was then quantified using an Opsys MR 96-well microtitre plate reader at 405 nm [9, 10]. The positive control was acarbose in dimethyl sulphoxide (DMSO). The percentage IC_{50} was calculated based using equation 1.

2.5.4 Phospholipase A_2 enzyme inhibition bioassay

The superfamily of phospholipase A_2 (PLA_2) enzymes key in fatty acid mobilization and signalling are abundant in nature (e.g. digestive juices, snake venoms and inflammatory cells). PLA_2s are interesting new targets for many therapeutic agents especially for managing inflammation based disorders including those of the nervous system [15, 16, 17]. The assay was done based on the colorimetric sPLA2 (type V) inhibitor screening assay (catalogue no. 10004883; Cayman Chemical) [15].

2.6 Statistical analysis

The computing and statistical analysis to compare the means as well as the standard errors of the IC_{50} values was performed using Microsoft Excel 2013 Windows version and SPSS® version 21.0 for Windows (IBM, Chicago, IL, USA). The level of significance was $p \leq 0.05$.

3. Results &Discussion

Fractions of the crude methanol extracts exhibited antimicrobial activities against various microorganisms (Table 1). *Erythrina abyssinica* leaf extract fractions exhibited the best antibacterial properties followed by those of *A. multiflorum*, *C. quadrangularis* leaf and then *C. quadrangularis* stem. The bark fractions of *E. abyssinica* showed the weakest antibacterial properties with only the ethanol and water fractions exhibiting significant activity against *E. coli* only. As can be seen in Table 1, the ethanol fraction exhibited good antibacterial activities on at least one bacteria for all plant extracts followed by petroleum ether, dichloromethane and lastly water. Plants have provided humans and animals with products for health and nutrition for centuries. They contain many phytoconstituents with several biological effects in complex matrixes. These phytoconstituents have wide ranging biological effects accounting for most of the traditional medicinal uses of those plants. Phenolics or polyphenols, such as cinnamic acids, benzoic acids, flavonoids, proanthocyanidins, coumarins, stilbenes, lignans and lignins are abundant as plant secondary metabolites and have wide ranging biological effects [6]. Their antioxidant properties are attributed to hydrogen donating capabilities that react with reactive oxygen and nitrogen species and chelation of metal ions key in free radical formation. Mechanisms of action of phenolics in biological systems is also thought to be based on their ability to interact with proteins thus inhibit various enzymes [6]. Apart from antioxidant properties; the enzyme inhibition (e.g. inhibition of various cytochrome P450 isoforms, lipoxygenases, cyclooxygenase and xanthine oxidase) can also explain the observed anti-inflammatory, antibacterial, antidiabetic, antifungal and antiviral properties of some plants (e.g. *C. quadrangularis*, *A. multiflorum* and *E. abyssinica*). Several scientist have demonstrated the existence of tannins, flavonoids, alkaloid, saponins and glycosides in alcoholic and/or aqueous extracts of *C. quadrangularis* aerial parts [7, 18, 19, 20]. Similar phytochemical profiles have been reported for *A. multiflorum* aerial parts as well as barks and leaves of *E. abyssinica* [7, 21, 22, 23].

All fractions for *C. quadrangularis* stem and *E. abyssinica* bark did not exhibit good activities against any of the chosen fungi (Table 1). *Adenium multiflorum* crude extract fractions exhibited the best antifungal activities followed by *E. abyssinica* fractions then *C. quadrangularis* leaf crude extract fractions. As can be also seen in Table 1, ethanol fractions, just like in antibacterial activities, exhibited good antifungal activities against all the chosen fungi. Dichloromethane fractions came in second followed by petroleum ether and lastly water which exhibited good activity against *Microsporum canis* (i.e. the *A. multiflorum* water fraction). Ethanol crude extract fractions of *E. abyssinica* and *A. multiflorum* had good antimicrobial properties against all the chosen bacteria and fungi chosen. Chloroform, water, methanol, butanol, ethyl acetate and/or hexane extracts or fractions of plants of the *Adenium* genus e.g. *A. obesum* exhibited moderate *in vitro* antibacterial activities against gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) supporting the observed antibacterial activities exhibited by *A. multiflorum* extracts or solvent fractions observed[24]. Similarly, extracts or solvent fractions have demonstrated significant antibacterial and fungal activities against bacteria (e.g. *E. coliB. subtilis* and *S. aureus*) and fungi (e.g. *Candida albicans* and *S. cerevisiae*) [25, 26]. Crude extracts of leaves and barks of *E. abyssinica* exhibited antimicrobial activities (e.g. against *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *S. typhi*) in several studies as well [27]. The exhibited antimicrobial properties may explain the applications of *C. quadrangularis*, *E. abyssinica* and *A. multiflorum* in animal wound healing. *Erythrina abyssinica* leaf and *A. multiflorum* crude extract fractions exhibited the best antimicrobial properties against the chosen six bacteria and four fungi. *Cissus quadrangularis* leaf extract fractions also exhibited good activity against most of the microorganisms chosen. Ethanol fractions exhibited the best activities against the chosen microorganisms.

As shown in Table 2, all methanol extracts and water fractions exhibited good anti-HIV reverse transcriptase activities relative to the positive controls used (Combivir® and Kaletra®). Most water fractions exhibited better inhibition (lower IC_{50}) especially for *C. quadrangularis* stem and *E. abyssinica* leaves followed by *E. abyssinica* bark then *A. multiflorum*. For the methanol crude extracts, *E. abyssinica* leaves showed the best activity (Table 2). Relative to the positive control and other extracts *E. abyssinica* leaves gave the best anti-tyrosinase activity followed by *E. abyssinica* bark then *A. multiflorum*. Both *C. quadrangularis* extracts exhibited the least activities based on the IC_{50} concentrations (Table 3). *Erythrina abyssinica* leaf and bark extracts gave the best anti-α-glucosidase activities (Table 4). *Cissus quadrangularis* leaf extracts also gave better activities relative to the positive control (acarbose) used. The rest (*C. quadrangularis* stem and *A. multiflorum*) exhibited similar

activities to the positive control (Table 4). Relative to the positive control, all the extracts exhibited good anti-phospholipase A_2 activities with *C. quadrangularis* leaf extracts exhibiting the best activities followed by *E. abyssinica* back, *A. multiflorum* and then *C. quadrangularis* stemextracts (Table 5). *Erythrina abyssinica* leaf extracts relative to other extracts exhibited the least anti-phospholipase A_2 activities though still showing better activities relative to the positive control (Table 5). Enzyme inhibition of herbal medicine can also explain several biological effects of many extracts justifying traditional applications. The abilities of phytochemicals to inhibit certain *in vivo* enzymes has many potential applications in modern medicine. These include the management of human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), asthma, chronic obstructive pulmonary disease (COPD), cardiovascular diseases, erectile dysfunction, gastrointestinal disorders, hepatitis B virus infection, hepatitis C virus infection, herpes virus infections and rheumatoid arthritis and/or related inflammatory diseases [28]. Plants or products thereof have exhibited fusion and revere trascriptase-1 inhibition activities among other anti-HIV effects examples include pine cone extract (YNS-PY-F) from *Pinusyunnanensis* and African potato extracts [11, 29]. All plants (*C. quadrangularis*, *E. abyssinica* and *A. multiflorum*) extract fractions demonstrated good activity against reverse transcriptase-1 offering possible hope for new compounds in HIV management. These plant crude extracts, in addition to interfering with reverse transcription, could also contain phytoconstituents which inhibit other unique enzymes and/or proteins in the HIV life cycle, such as those involved in virus entry, integrase or protease. These phytochemicals could be ribosome inactivating proteins, alkaloids, flavonoids, lignans and polysaccharides, some of which have been shown to be present in the plants as highlighted above [29]. Extracts from seeds of *E abyssinica* were also found to possess good anti-HIV activities, among other effects, in Sudan [30], supporting the findings of this study. Many plant extracts including *E. abyssinica* have also shown activity against tyrosinase enzyme, a biological effect key in depigmentation and cosmeceuticals products [31, 32, 33]. Furthermore, many plants have traditional and potential applications in diabetes and lipid disorder management based on their phytochemical contents. Many plant crude extracts have shown activity against α-glucosidase [34, 35]. *Cissus quadrangularis* extracts have also exhibited activities against many enzymes such as α-glucosidase, amylase and lipase in other *in vitro* assays [36], further supporting the findings of this study. *Erythrina abyssinica*, *A. multiflorum* and *C. quadrangularis* extracts possess good antimicrobial activities against some bacteria and fungi known to inhabit the skin and wounds thus justifying folklore uses in animal wound management. Apart from demonstrating and validating the wound healing uses, the study has also shown that the plant extracts have many other potential medicinal applications e.g. in bacterial/fungal infections, HIV/AIDS management, depigmentation and diabetes and lipid disorders control/management.

3.1 Tables

Table 1: Antibacterial and antifungal properties of extract fractions of some plants used in animal wound healing

Scientific name	Plant part	Extract	Bacterial MIC (mg/ml)						Fungal MIC (mg/ml)			
			B.s	S.a	E.c	K.p	B.a	S.e	C.a	T.m	T.t	M.c
		PE	0.39	1.562	1.562	6.25	12.5	6.25	6.25	1.562	3.125	3.125
Cissus quadrangularis	Leaf	DCM	0.781	1.562	3.125	0.781	12.5	6.25	1.562	1.562	0.781	0.781
		Ethanol	0.098	0.781	0.098	1.562	0.39	0.39	3.125	1.562	1.562	0.781
		Water	1.562	3.125	0.781	6.25	1.562	6.25	1.562	12.5	1.562	6.25
		PE	6.125	12.5	6.125	3.125	6.25	6.25	12.5	3.125	1.562	1.562
Cissus quadrangularis	Stem	DCM	6.125	12.5	12.5	6.25	6.25	6.25	6.25	3.125	3.125	6.25
		Ethanol	3.125	3.125	0.098	1.562	0.195	0.781	1.562	1.562	6.25	6.25
		Water	1.562	12.5	1.562	3.125	1.562	6.25	6.25	3.125	1.562	3.125
		PE	0.781	0.195	0.39	0.098	6.25	1.562	0.781	3.125	6.25	1.562
Erythrina abyssinica	Leaf	DCM	0.39	0.098	0.781	0.098	3.125	1.562	0.39	1.562	6.25	1.562
		Ethanol	0.098	0.781	0.098	0.781	0.39	0.781	0.781	0.781	0.781	0.781
		Water	3.125	3.125	0.098	3.125	3.125	1.562	1.562	1.562	3.125	0.781
		PE	3.125	1.562	6.125	3.125	12.5	6.25	6.25	6.25	3.125	6.25
Erythrina abyssinica	Bark	DCM	3.125	1.562	12.5	12.5	12.5	6.25	3.125	6.25	3.125	6.25
		Ethanol	6.125	3.125	0.098	3.125	3.125	1.562	1.562	1.562	3.125	1.562
		Water	3.125	12.5	0.098	6.25	6.25	6.25	1.562	3.125	6.25	12.5
		PE	0.098	0.781	0.098	0.39	0.39	6.25	0.39	0.781	1.562	1.562
Adenium multiflorum	Aerial	DCM	0.098	6.125	12.5	1.562	3.125	0.39	1.562	1.562	0.781	0.781
		Ethanol	0.098	0.098	0.098	0.781	0.098	0.781	0.39	0.39	0.39	0.39
		Water	6.125	6.125	3.125	1.562	3.125	1.562	3.125	1.562	1.562	0.781
Neomycin			1.6×10^{-3}	0.8×10^{-3}	0.8×10^{-3}	1.6×10^{-3}	0.8×10^{-3}	0.8×10^{-3}				
Amphotericin B									9.8×10^{-3}			

Extracts-Petroleum ether (PE), Dichloromethane (DCM) and Ethanol (70% v/v). Bacterial cultures-*Bacillus subtilis* (B.s), *Staphylococcus aureus* (S.a), *Escherichia coli* (E.c), *Klebsiella pneumoniae* (K.p), *Brevibacillus agri* (B.a) and *Staphylococcus epidermis* (S.e). Fungal cultures-*Candida albicans* (C.a), *Trichophyton mentagrophytes* (T.m), *Trichophyton tonsurans* (T.t) and *Microsporum canis* (M.c). Plant extracts with MIC values highlighted in bold are considered to have very good activity (MIC <1 mg/mL).

Table 2: Anti-human immune virus (HIV) activities of extracts of some plants use in animal wound management

Medicinal Plant	Plant Part	Anti-HIV activity IC_{50} (mg/mL)	
		Extracts	
		Methanolic	Water
Cissus quadrangularis	Leaves	0.0354±0.0122[c]	0.0302±0.0098[c]
Cissus quadrangularis	Stem	0.0384±0.0062[c]	0.0115±0.0013[a]
Erythrina abyssinica	Leaves	0.0199±0.0004[b]	0.0150±0.0121[a]
Erythrina abyssinica	Bark	0.0417±0.0236[d]	0.0179±0.0024[b]
Adenium multiflorum	W/plant	0.0312±0.0086[c]	0.0194±0.0087[b]
Combivir®			0.0650±0.0031[e]
Kaletra®			0.3301±0.1050[f]

MeOH-methanol, values with different letters are significantly different at $p<0.05$; n=2.

Table 3: Anti-tyrosinase activity of methanol extracts some plants used in animal wound management

Medicinal Plant	Plant Part	Tyrosinase IC_{50} (µg/ml)
Cissus quadrangularis	Leaves	98.44±2.16[e]
Cissus quadrangularis	Stem	111.71±2.35[ef]
Erythrina abyssinica	Leaves	36.11±2.96[b]
Erythrina abyssinica	Bark	41.94±3.76[bc]
Adenium multiflorum	W/plant	66.24±3.32[d]
Kojic acid		19.38±2.72[a]

Values with different letters are significantly different at $p<0.05$; n=3. The lower the inhibition concentration (IC_{50}) the better the activity of the crude extracts.

Table 4: Anti-α-glucosidase activity of extracts some plants used in animal wound management

Medicinal Plant	Plant Part	Glucosidase IC_{50} (µg/ml)
Cissus quadrangularis	Leaves	69.91±2.296[b]
Cissus quadrangularis	Stem	81.22±0.041[c]
Erythrina abyssinica	Leaves	53.50±0.064[a]
Erythrina abyssinica	Bark	54.31±1.779[a]
Adenium multiflorum	W/plant	87.81±2.358[c]
Acarbose		83.60±2.600[c]

Values with different letter are significantly different at $p<0.05$; n=2. Results are expressed as means± standard errors of two independent experiments, each experiment in duplicate.

Table 5: Anti-phospholipase A_2 (PLA2) activity of extracts some plants used in animal wound management

Medicinal Plant	Plant Part	IC_{50} (mg/ml)
Cissus quadrangularis	Leaves	0.035±0.006
Cissus quadrangularis	Stem	0.139±0.006
Erythrina abyssinica	Leaves	1.395±0.098
Erythrina abyssinica	Bark	0.059±0.001
Adenium multiflorum	W/plant	0.077±0.012
Thioetheramide PC		4.552±0.226

Cissus quadrangularis leaf extracts exhibited the best anti-phospholipase A_2 activity and *E. abyssinica* leaf extracts the least.

3.2 Equations

$$\text{Percentage inhibition } (\%) = \frac{\text{Ac}-\text{As}}{\text{Ac}} \text{X } 100 \quad (1)$$

Equation 1: Ac-absorbance of the control, As-absorbance of the sample preparation. The IC_{50} was determined in duplicate for each sample and presented as means± standard errors of micrograms per ml that give good (≥70%) inhibition.

4. Conclusions

Crude extract fractionsof *Erythrina abyssinica* leaf and *A. multiflorum* aerial parts exhibited the best antimicrobial activities. *Cissus quadrangularis* leaf fractions also exhibited good antimicrobial properties followed by *C. quadrangularis* stem extract fractions. *Erythrina abyssinica* bark fractions gave the least antimicrobial activities. Ethanol solvent fractions demonstrated best activities against both the bacteria and fungi chosen. All methanol and water fractions of all the plants exhibited good anti-HIV reverse transcriptase -1 activities. *Erythrina abyssinica* leaves gave the best anti-tyrosinase activity followed by *E. abyssinica* bark then *A. multiflorum*. Crude extracts of *E. abyssinica* leaf and bark gave the best anti-α-glucosidase activities and *C. quadrangularis* leaf also gave good activity. *Cissus quadrangularis* leaf extracts exhibited the best anti-phospholipase A_2 activities followed by *E. abyssinica* bark, *A. multiflorum* and *C. quadrangularis* stem extracts in that order. *Erythrina abyssinica* leaf extracts exhibited the least anti-phospholipase A_2 activities.

5. Conflict of interest

No conflicts of interest to declare.

6. Author Contributions

A.M. G.M. S.K. T.M. and A.R.N conceptualized and developed the research as well as experiments. A.M. T.D.C. I.M. T.D. T.M. and A.R.N. conducted the experiments and analysis of results. All authors contributed to the drafting, proofreading and approval of the manuscript.

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