Tanzania Journal of Science 47(1): 57-69, 2021
ISSN 0856-1761, e-ISSN 2507-7961

Isolation and Quantification of Flavonoids from *Kigelia africana* (Lam.) Benth Fruit and Bark and their Antiradical and Antibacterial Activities

Pamhidzai Dzomba* and George Mhini
Department of Chemistry, Faculty of Science and Engineering, Bindura University of Science Education, P Bag 1020, Bindura, Zimbabwe
**Corresponding author email: pdzomba@gmail.com; pdzomba@buse.ac.zw*
ORCID ID: http://orcid.org/0000-0001-6821-2606
Co-author e-mail: mhinigrorge@gmail.com
Received 2 Oct 2020, Revised 12 Dec 2020, Accepted 28 Dec 2020, Published Feb 2021
https://dx.doi.org/10.4314/tjs.v47i1.6

Abstract

Kigelia africana is an evergreen deciduous tree used in Zimbabwe folklore medicine for wound and diabetes ulcers healing. This paper reports the isolation and quantification of flavonoids from stem barks and fruits of *K. africana* as well as their antibacterial and antiradical activities. Flavonoids in ethanol and ethyl-acetate extracts were quantified using UV-Vis spectrophotometry and were found to be 213.490 ± 0.001 mg/L and 36.614 ± 0.001 mg/L, respectively per quercetin equivalent. The preparative and analytical thin layer chromatography was used to profile the flavonoids from fruits and barks using ethyl-acetate and petroleum ether (3:7 v/v) mobile solvent systems. Ethanol extract yielded three flavonoids with R_f values of 0.05; 0.79 and 0.93, while the ethyl-acetate extract yielded two flavonoids with R_f values of 0.21 and 0.73. The antiradical activities of the extracts were determined using DPPH radical scavenging method using quercetin as reference, and the activities were found to be over 80% at 150 mg/L. The disc diffusion method was used for antibacterial activity determination, and flavonoids from both ethanol and ethyl-acetate extracts showed zones of inhibition ranging from 0 to 22 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Although the present study revealed that flavonoids from *K. africana* possess significant antibacterial and antioxidant activities, more studies are necessary for them to be incorporated in polyherbal formulations for wound and diabetic ulcer topical treatments.

Keywords: Diabetic foot ulcers; *Kigelia africana*; Antibacterial activity; Flavonoids; Antioxidant activity.

Introduction

Diabetic ulcers is a major problem in diabetic patients with limited treatment regimes conventionally (Lipsky et al. 2012). Untreated wounds develop into gangrenes leading to amputation. Infected diabetic foot ulcers account for approximately 25% of all hospital admissions for diabetes patients (Citron et al. 2007). Diabetes patients have impaired micro-vascular circulation causing treatments of diabetes wounds to be difficult (Lipsky et al. 2012). This is because phagocytic cells are fewer at the affected tissues combined with poor nutrient availability (Lipsky et al. 2005, 2012). *Staphylococcus aureus* is the most frequent pathogen on diabetic foot ulcers followed by various *Pseudomonas* species (Dang et al. 2003). Thus an effective diabetic ulcers remedy should have antiradical and antibacterial properties. Multidrug resistance

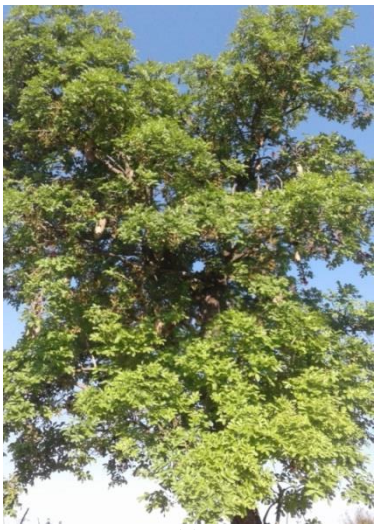
and costs of conventional medicines complicates treatments of diabetic ulcers in many patients especially in developing countries (Lipsky et al. 2012, Mishra et al. 2013). This has led to the search for new agents mainly phytochemicals from plants that can be drug alternatives (Atta et al. 2017).

Herbal medicines are common nowadays due to their availability and low costs. The presence of bioactive components in plants particularly phenolic acids and flavonoids enable plants to possess anti-oxidative and many pharmacological properties (Ignat et al. 2011, Goveas and Abraham 2013). In folklore medicines in Zimbabwe, people use *Kigelia africana* as a remedy to wounds and diabetes ulcers. The local people use the bark, root or fruit powders or their water infusions to treat burns, general wounds and diabetic ulcers (Saini et al. 2009). The plant is administered topically on the wound or diabetic ulcer. The stem and root barks and fruits have a rich yellowish colour implying richness in flavonoids (Saini et al. 2009). The *K. africana* plant is rich in various phytochemicals such as flavonoids, alkaloids and steroids, thus may have significant medicinal properties (Saini et al. 2009). Therefore, the present study was designed to investigate the presence of antibacterial and antiradical flavonoids in the fruits and barks of *K. africana*. Flavonoids, abundant in fruits and barks of medicinal plants, have been studied extensively as they possess high antioxidant and antibacterial properties against both Gram positive and Gram negative bacteria (Saini et al. 2009, Stalikas 2007).

Materials and Methods

Sample collection

The mature fruits and barks were authenticated by a taxonomist at Harare Botanical garden. The samples of *K. africana* fruits and barks (Figure 1) were collected from the forests in Mukore and Mashoko areas in Bikita district, Masvingo Province, Zimbabwe in December 2018 and a voucher specimen 2018/12 was deposited in the Chemistry Department of Bindura University of Science Education.



(a)

(b)

(c)

Figure 1: (a) *K. africana* tree, (b) its mature fruit and (c) chopped barks and fruits.

Sample treatment

The collected barks and fruits of the *K. africana* plant were chopped into small pieces and air dried to prevent enzymatic actions. This was followed by crushing and grinding into fine powder and then stored for further extraction processes.

Instruments

Electronic analytical balance AE Adam was used for measuring the masses of the samples. The Labotec horizontal shaker was used for shaking the volumetric flasks containing the extracts. Solid phase extraction (SPE) cartridges packed with silica gel were used for concentrating the crude extracts after solid liquid extraction. RE rotary evaporator manufactured by Xian Heb Biotechnology company was used for concentrating the extracts from the SPE. The TLC plates used were supplied by Merck Darmstadt, Germany. Stuat Scientific magnetic stirrer hot plate was used for heating.The KnF Neuberger vacuum pump was used for enhancing the filtration process. UV-Vis Thermo Scientific Genesys 10S spectrophotometer with Visionlite software was used for the determination of flavonoids content in the extracts. A CE Serial No. 15102295 Vilber Lourmat UV-Visualization detector set at 365 nm was used for visualising the TLC chromatograms.

Chemical and reagents

The methanol, ethanol, acetic acid, chloroform, hexane, petroleum ether, ethyl-acetate (analytical grades) were supplied by Sky Labs, South Africa. The Mueller Hinton Agar used in this study was supplied by Biomark Laboratories, India. Streptomycin discs were supplied by Mast Diagnostics, UK. The quercetin was supplied by Sigma Aldrich, Germany. The 2, 2 diphenyl-1-picrylhydrazyl (DPPH, analytical grade) used was supplied by Sigma Aldrich, Germany, Lot Number STBC5113V. Mupirocin a topical antibiotic cream was bought from local pharmacies.

Solvent extraction

Solvent extraction was performed according to the method of Gwatidzo et al. (2018) with minor modifications.

Liquid solid extraction using ethanol and ethyl-acetate

A mass of 10 g of powdered *K. africana* fruits and barks was mixed with 50 mL of absolute ethanol, analytical grade in a 250 mL volumetric flask. The sample was shaken for 30 minutes on a horizontal shaker and then filtered using Whatman's number 1 filter paper. The filtrate was collected for further analysis. The maceration process was repeated twice more with further 30 mL and 20 mL absolute ethanol. The collected filtrates for the whole process were mixed to obtain the ethanol extract.

The extraction procedure was repeated using ethyl-acetate. The two extracted samples were concentrated using solid phase extraction process and rotary evaporation (Gwatidzo et al. 2018, Dzomba and Muchanyereyi 2012)**.**

Solid phase extraction

The solid phase extraction was done according to the methods of Mumin et al. (2006) and Gwatidzo et al. (2018) with minor modifications. Each extract from liquid solid extraction was taken to the solid phase extraction process. This was done to get concentrated crude extracts. The SPE cartridge was conditioned by allowing 5 mL of absolute ethanol to pass through by the help of gravity. Equilibration was achieved by passing 5 mL of distilled water through the silica gel sorbent. The ethanol extract was then loaded and passed through the cartridge under gravity. 1.5 mL of acetone was used in order to maximise sample clean-up. The final stage was eluting using 5 mL methanol and concentrated on a rotary evaporator at 40 °C. The collected samples were then stored for further analysis. The procedure was repeated using ethyl-acetate extract.

Analytical thin layer chromatography

Thin layer chromatography (TLC) was done according to the methods of Gu et al. (2009 and Gwatidzo et al. 2018) with minor modifications. TLC plates, 10 x 5 cm silica gel were used for the chromatographic analyses. The plates were first activated through heating at 100 °C for about 10 minutes and then allowed to cool to about 25 °C. Pencil drawn baseline which was 1.5 cm was drawn from the edges of the TLC plates. The two extracts were placed on the pencil drawn baseline of different TLC plates using thin capillary pipettes. The plates were placed in a development chamber containing a trial solvent system. Several trial runs were carried out in order to determine the mobile phase which gave the best separation. The following solvent systems were used initially in order to search for the best mobile phase.

1. Ethanol: acetic acid: water (6:11:3 v/v/v);
2. Chloroform: methanol: hexane (2:7:1 v/v/v);
3. Methanol: acetic acid: water (2:2:6 v/v/v);
4. Ethyl-acetate: petroleum ether (3:7 v/v); and
5. Water: ethyl acetate: methanol (1.5:10:2 v/v/v).

The ethyl-acetate: petroleum ether (3:7 v/v) solvent system gave the best separation. The solvent front was allowed to move until it reached the position which was 1.5 cm from the top end of the TLC plate. The plate was removed and the position of the solvent front was marked using a soft pencil. The plate was air dried and then sprayed with 1% ethanolic aluminium chloride revealing reagent and then dried again. The dried TLC plates were visualised under UV/Vis lamp at 365 nm. The positions of the spots formed after spraying the ethanolic aluminium chloride revealing solution were identified and marked and finally the retention factor of each spot was calculated.

Preparative thin layer chromatography

Silica gel TLC plates were used. A concentrated band of ethanol or ethyl acetate extracts was deposited on a pencil drawn baseline on the TLC plates which was about 1.5 cm from the edge and the plates were allowed to dry at room temperature. The dried plant extract samples were gently placed in a development glass chamber with the mobile phase ethyl-acetate: petroleum ether (3:7 v/v). The solvent travelled almost the ¾ length of the plates and the final position of the solvent front was identified and marked by a soft pencil. The plates were left to stand at room temperature until they were dried. The extracts were separated into bands and bands which tested positive for the flavonoids (see Figure 2) were scratched and re-dissolved in 2 mL of ethanol separately and then filtered, collected and stored in a fridge (0–5 °C) waiting for further analysis. 1% ethanolic aluminium chloride solution was used for testing for the presence of flavonoids. The positions of different bands were identified and the retention factor values of these bands were calculated.

Quantitation of flavonoids

Exactly, 2 mL of the extracts were mixed with 0.1 mL of aluminium chloride and 0.1 mL of sodium acetate and then incubated at room temperature for 30 minutes. The intensity of the colour of extracts formed after incubation was measured at 415 nm. The procedure was repeated for other flavonoids extracts. A calibration curve for quercetin (15–300 mg/L) was plotted from which the flavonoids content of the extracts was determined as per quercetin equivalent.

Antiradical activity determination

The DPPH (2, 2 diphenyl-1-picrylhydrazyl) antiradical activity determination method of Muchuweti et al. (2013) was followed with minor modifications.

***Preparation of sample solution*:** 0.1 mM solution of DPPH was prepared by dissolving 0.0039 g in 100 mL of methanol. A 3 mL volume of this methanolic solution of the DPPH was placed into a cuvette and 20 microlitres of the sample added. Absorbance at 517 nm was read on a UV-Vis

spectrophotometer over 20 min. Concentration ranging from 15 to 300 mg/L were prepared.

***Preparation of standard solution*:** 20 mL of methanol was used initially to dissolve 0.15 g of quercetin and a stock solution with concentration of 300 mg/L was prepared. Five standards having concentration ranging from 15 mg/L up-to 300 mg/L were prepared from the stock solution using serial dilutions.

Antibacterial activity determination

The combinations of standardised single disk and well diffusion methods of Dzomba et al. (2012) and Sen and Batra (2012) with minor modifications were followed.

***Sample preparation*:** Mueller Hinton Agar (MHA) (M173) plates were used for growing *S. aureus* and *P. aeruginosa*. 38 g of MHA were dissolved in 1000 mL of distilled water and boiled to dissolve the medium completely. The medium was sterilised at 121 °C for 15 minutes in an autoclave and plates were prepared with a depth of about 4 mm. The media was left to solidify and the bacteria cultures were placed on the agar plate individually and spread evenly using a sterile bent glass rod over the entire surface. The wells were prepared using cork borers of diameter 5 mm under aseptic conditions. Exactly 50 mL of ethanol were used to dissolve 0.15 g of flavonoids in order to prepare a stock solution of concentration 300 µg/L, four samples having concentrations ranging from 10 µg/L to 300 µg/L were prepared from the stock solution using serial dilutions. The Mueller Hinton agar plates were incubated for 16-18 hours at 35 °C and the zones of the inhibition were measured using a 30 cm ruler. The zones indicated the susceptibility or resistance of a bacterium to the extract. The zones of inhibition diameters of the extracted flavonoids were compared with the zone of inhibition diameter obtained when 300 µg mupirocin discs of diameter size 5 mm were used. Absolute ethanol and sterile water were used as negative control solutions. The antibiotic mupirocin 300 µg/L was used as a positive control.

Statistical Analyses

The results are presented as mean ± standard deviation. All the statistical analyses between treatments were determined at 95% significance level using ANOVA and Student's T test. The statistical analyses were computed using IBM SPSS 20 software.

Results

Extraction results

Plant samples, fruits and barks were stored and dried in a refrigerator at 5 °C in order to improve its shelf life and reducing sample degradation (Doughari 2012). In this study, the flavonoids were extracted using absolute ethanol and ethyl-acetate. Table 1 shows the quantities obtained after extracting with ethanol and ethyl acetate solvents as per 10 g samples used. Table 1 revealed that 1.07 ± 0.12 g and 0.65 ± 0.06 g were obtained from extracting 10 g of powdered fruits and barks of *K. africana* using ethanol ethyl-acetate, respectively.

Table 1: Quantities of the extract

Extract	Sample	Mass of extracts obtained (g) per 10 g of sample used
Ethanol	1	1.0012
	2	1.2100
	3	1.0014
	Average	1.07 ± 0.12
Ethyl acetate	1	0.7116
	2	0.5938
	3	0.6345
	Average	0.65 ± 0.06

Thin layer chromatography results

Table 2 shows the results obtained when the chromatograms were sprayed with a visualising reagent. The visualising reagent used was 1% ethanolic aluminium chloride. The spots which gave positive tests for flavonoids were designated ++ and those spots which tested negative were designated x. The retention factor values were calculated using the equation below.

$$\text{Retention factor } (R_f) = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

Table 2: Flavonoids profiling

Type of extract	Number of spots	Retention factor values	Test on flavonoids
Ethanol extract	10	0.05	++
		0.15	x
		0.23	x
		0.27	x
		0.41	x
		0.62	x
		0.68	x
		0.79	++
		0.89	x
		0.93	++
Ethyl-acetate	6	0.13	x
		0.21	++
		0.33	x
		0.45	x
		0.56	x
		0.73	++

KEY: ++ Flavonoids tested positive using ethanolic aluminium chloride;
x Flavonoids tested negative using ethanolic aluminium chloride.

Analytical and Preparative TLC

The chromatograms on Figure 2 show the spots which tested positive for flavonoids after spraying with the revealing agent 1% ethanolic $AlCl_3$ and dried in an oven and then visualised under UV-Vis at 365 nm. The ethanol extract gave 3 spots which tested positive for the flavonoids and they gave characteristic blue, yellow and orange colours (R_f 0.05 = yellow; R_f 0.93 = orange; R_f 0.79 = yellow). The ethyl acetate extract gave two spots which tested positive for flavonoids and they both have a characteristic yellow colour (Kay et al. 2012, Mohammed 1996).

Quantitation of flavonoids

Extracts from *K. africana* showed presence of flavonoids with a maximum wavelength of 426 nm which coincided with that of quercetin (Figure 3). Flavonoids have aromatic rings, hence the conjugated double bonds allow them to be analysed in ultraviolet or visible ranges. Interferences from other phytochemicals are reduced when complexes with bathochromic effect are formed as a result of the dative bonds formation between aluminium cations and the flavonoids (Silva et al. 2009). Ethanol extract showed higher amounts of flavonoids than ethyl acetate extract (Table 3).

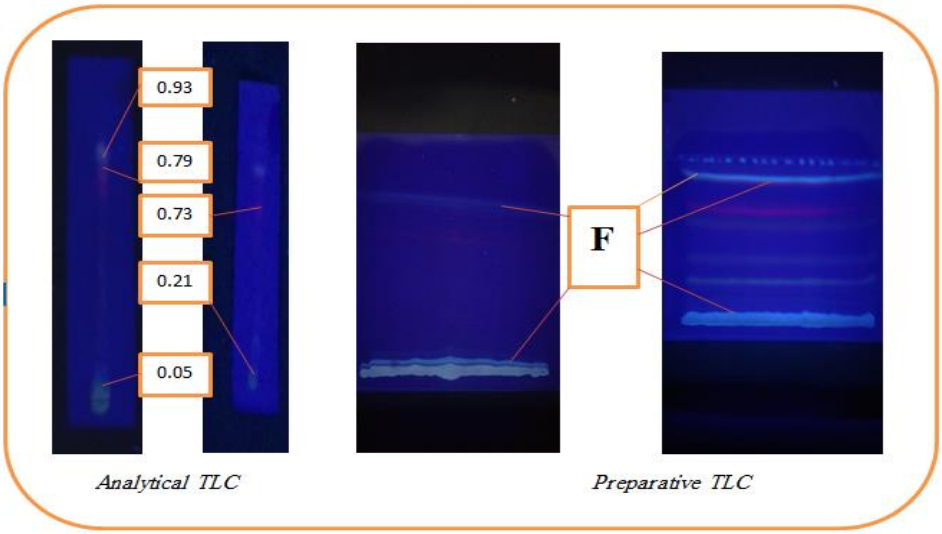


Figure 2: TLC chromatograms showing flavonoids after using revealing agent.

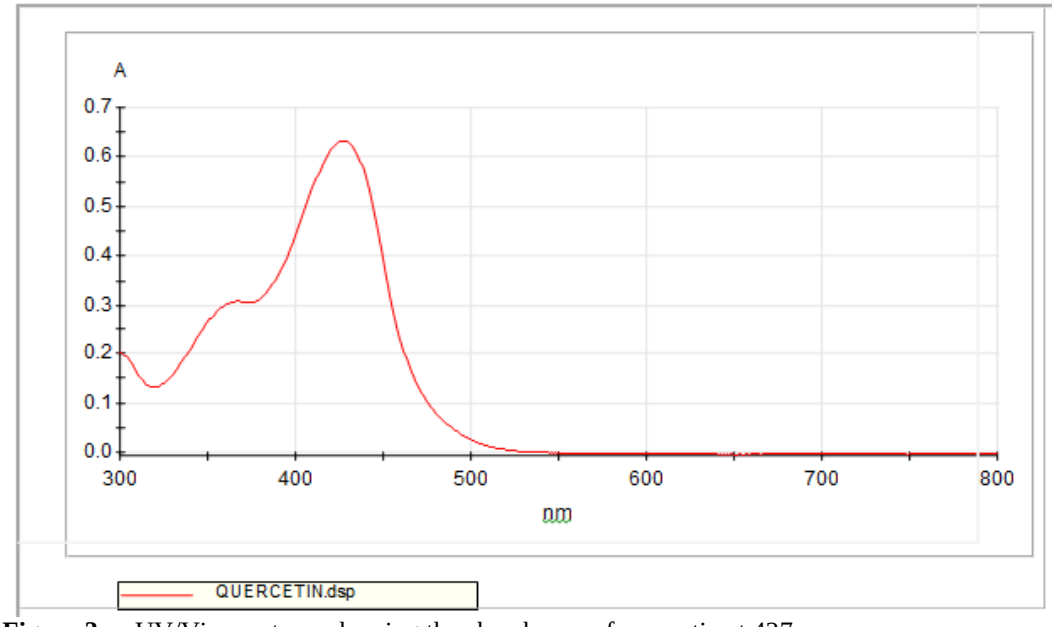


Figure 3: UV/Vis spectrum showing the absorbance of quercetin at 427 nm.

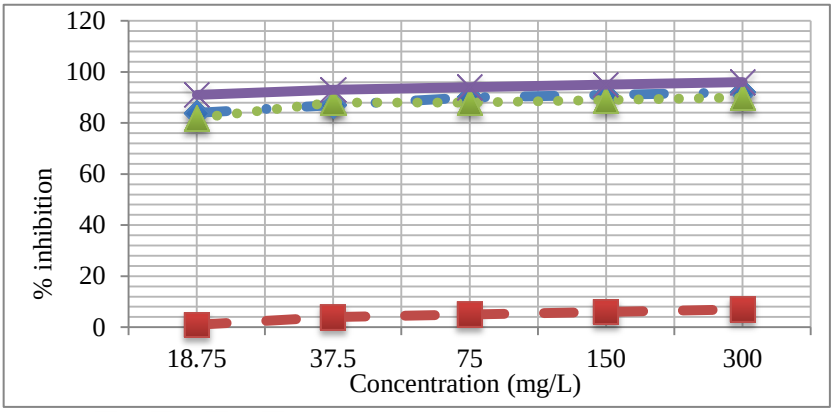
Table 3: Concentrations of flavonoids per quercetin equivalent extracted

Extract	Absorbance			Average	Concentration (mg/L)
Ethanol	0.862	0.861	0.862	0.862	213.490 ± 0.001
Ethyl acetate	0.392	0.392	0.391	0.392	36.614 ± 0.001

Antiradical activity determination

Ethanol extract

The antiradical activity of flavonoids (R_f = 0.05; R_f = 0.79 and R_f = 0.93) obtained from ethanol extract of *K. africana* versus quercetin standard can be modelled by the following graph in Figure 4.

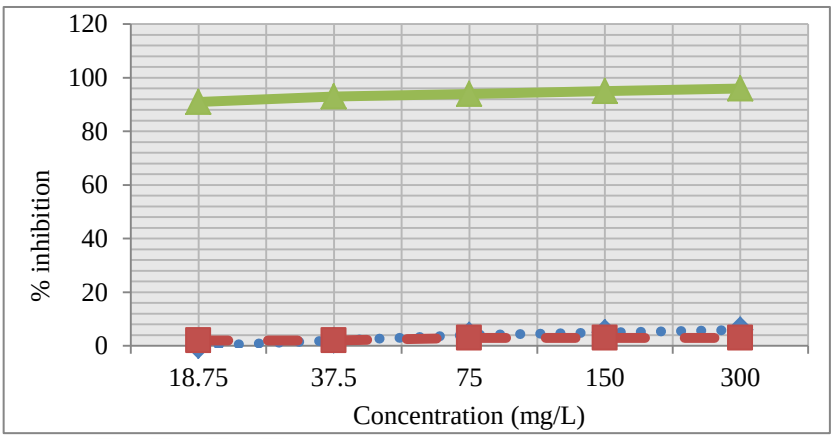


Key: Flavonoids with R_f of 0.79; Flavonoids with R_f of 0.93; Flavonoids with R_f of 0.05; Quercetin standard.

Figure 4: Graph showing antiradical activity of flavonoids from ethanol extract versus quercetin standard.

Ethyl acetate extract

The antiradical activity of flavonoids (R_f = 0.21; R_f = 0.73) obtained from ethyl-acetate extract of *K. africana* versus quercetin standard can be modelled on Figure 5.



Key: Flavonoids with R_f of 0.73; Quercetin standard; Flavonoids with R_f of 0.21.

Figure 5: Graph showing antiradical activity of flavonoids from ethyl-acetate extract versus quercetin standard.

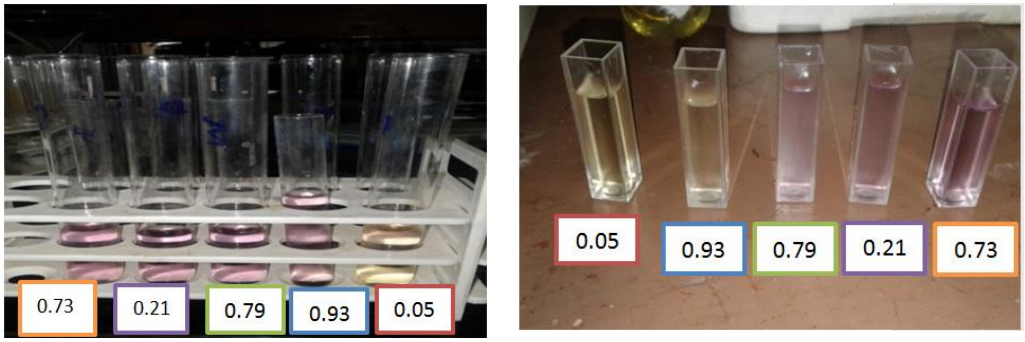


Figure 6: Photographs showing colours obtained with DPPH assays of flavonoids extracted using ethanol.

Figures 4-6 show the results of antioxidant activity analysis (DPPH) tests for the ethanol and ethyl-acetate extracts of *K. africana*. The yellow colour indicates high antioxidant activity and purple colour indicates lack of antioxidant activity. The results showed that for ethanol extract flavonoids separating at R_f; 0.05, 0.93 Table 4 and Figure 4 exhibited DPPH antiradical activity that was comparable to that of quercetin. Flavonoid separating at 0.79 consisted of antiradical activity; however, it was lower than that of quercetin.

Table 4: The IC_{50} values of isolated flavonoids and quercetin

Sample	IC_{50} (mg/L)
Flavonoids R_f 0.05	11.0
Flavonoids R_f 0.79	520.8
Flavonoids R_f 0.93	11.2
Flavonoids R_f 0.21	2343
Flavonoids R_f 0.73	468.8
Quercetin	10.3

Antibacterial activities

Figure 7 and Tables 5 and 6 show results of the antibacterial activities of the flavonoids and solvents as isolated by preparative TLC. Agar well diffusion method was regarded as a relatively low sensitive assay as samples were further diluted when diffusing into the agar. As a result, it was combined with disc diffusion in this study. The diameters of zones of inhibition were measured according to Uddin et al. (2007).

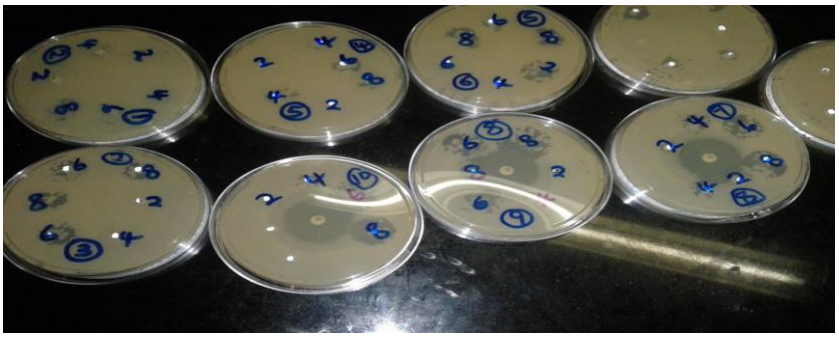


Figure 7: Pictures showing zones of inhibition.

Table 5: Zones of inhibition shown by the isolated flavonoids

	Diameter of zone of inhibition (mm)			
Amount of flavonoids of R_f value of 0.05	37.5 µg/mL	75 µg/mL	150 µg/mL	300 µg/mL
Staphylococcus aureus	2 ± 0.01	4 ± 0.01	17 ± 0.01	19 ± 0.01
Pseudomonas aeruginosa	0	1 ± 0.01	10 ± 0.001	18 ± 0.01
Amount of flavonoids of R_f value of 0.21	37.5 µg/mL	75 µg/mL	150 µg/mL	300 µg/mL
Staphylococcus aureus	0	2 ± 0.01	10 ± 0.01	12 ± 0.01
Pseudomonas aeruginosa	0	1 ± 0.01	3 ± 0.01	6 ± 0.01
Amount of flavonoids of R_f value of 0.73	37.5 µg/mL	75 µg/mL	150 µg/mL	300 µg/mL
Staphylococcus aureus	2 ± 0.01	4 ± 0.01	7 ± 0.01	9 ± 0.01
Pseudomonas aeruginosa	0	0	4 ± 0.01	9 ± 0.01
Amount of flavonoids of R_f value of 0.79	37.5 µg/mL	75 µg/mL	150 µg/mL	300 µg/mL
Staphylococcus aureus	1 ± 0.01	7 ± 0.01	14 ± 0.01	18 ± 0.01
Pseudomonas aeruginosa	1 ± 0.01	4 ± 0.01	7 ± 0.01	11 ± 0.01
Amount of flavonoids of R_f value of 0.93	37.5 µg/mL	75 µg/mL	150 µg/mL	300 µg/mL
Staphylococcus aureus	2 ± 0.01	4 ± 0.01	19 ± 0.01	22 ± 0.01
Pseudomonas aeruginosa	1 ± 0.01	2 ± 0.01	10 ± 0.01	17 ± 0.01
Mupirocin	37.5 µg/mL	75 µg/mL	150 µg/mL	300 µg/mL
Staphylococcus aureus	2 ± 0.01	4 ± 0.01	18 ± 0.01	23 ± 0.01
Pseudomonas aeruginosa	1 ± 0.01	2 ± 0.01	10 ± 0.01	16 ± 0.01

Table 6: Control samples zones of inhibition

	Diameter of zone of inhibition (mm)	
Control	Water	99.9% ethanol
Staphylococcus aureus	0	1
Pseudomonas aeruginosa	0	1

Discussion

Extraction

Plant samples were stored in a refrigerator at 5 °C in order to increase its shelf life by reducing sample degradation (Doughari 2012). In this study, the flavonoids were extracted using absolute ethanol (99.9%) and ethyl-acetate. Table 1 reveals that 1.07 ± 0.12 g were obtained from extracting 10 g of powdered fruits and barks of *K. africana* using ethanol and also 0.65 ± 0.06 g were obtained after using ethyl-acetate as an extracting solvent. These results showed that extraction using ethanol gave a considerable amount of extracts. The high efficiency of ethanol as an extracting solvent is attributed to its intermediate polarity which enables it to extract polar and apolar compounds (Khandelwal et al. 2016).

Flavonoids profiling

The study revealed the presence of flavonoids in the fruits and barks of *K. africana* tree. The identification of the flavonoids on TLC chromatograms was applicable after spraying the bands with ethanolic aluminium chloride which is the revealing agent. The sprayed TLC

chromatograms were then viewed under UV-Vis spectrophotometer at 365 nm. In this study, a total of five different flavonoid types were observed as shown in Figure 2. Spots which gave negative results after using the revealing agent (1% ethanolic aluminium chloride) were regarded as the unknowns and discarded. The ethanol extract yielded three types of flavonoids having the R_f values of 0.05, 0.79 and 0.93. The ethyl-acetate extract contained two types of flavonoids with retention factor values of 0.21 and 0.73. Ethanol was a solvent used in this study for re-dissolving the isolated flavonoids because it could dissolve all the actives contained in the sample. Previously, *K. africana* was found to contain two flavonoids, namely quercetin and luteolin (Dennis and Wulandari2017, Saini et al. 2009). According to Doughari (2012), Mohammed (1996) and the TLC results, the flavonoids at R_f 0.05 showing yellow colour and at R_f 0.93 showing orange colour are flavonol glycosides, quercetin, myricetin, kaempferol and luteolin 7-o-glycosides.

Antiradical activity

Table 4 showed that the flavonoids from ethanol extract produced IC_{50} values of 11 µg/L, 11.2 µg/L and 520.8 µg/L, while those from ethyl acetate extract produced IC_{50} values of 468.8 µg/L and 2343 µg/L. The extracts converted DPPH free radical into DPPHH (Dzomba et al. 2012) resulting in the change of colour from purple to yellow as revealed in Figure 6. The heterocyclic nature of flavonoids enable antioxidant activity by allowing conjugation between hydroxyl groups and the aromatic rings (Kumar and Pandey 2013). The study is in agreement with the previous study which showed the presence of antioxidants in ethanol extract of *K. africana* (Olalye and Rocha 2007). Flavonoids obtained by ethanol extraction were also more effective free radical scavengers (Mohdaly et al. 2009).

Antibacterial activities

Crude extracts of *K. africana* are used in folklore medicines to treat wounds and diabetic ulcers, inflammation and sores (Saini et al. 2009). Flavonoids form chemical complexes with proteins due to the presence of hydrogen bonds and hydrophobic effects thereby inactivating microbial adhesions and cell walls (Kumar and Pandey 2013). Flavonoids reduce the fluids present in membranes of bacterial cells resulting in the death of bacteria (Kumar and Pandey 2013). The diameters of zones of inhibition were measured according to Uddin et al. (2007). Agar well combined with disc diffusion showed that, *K. africana* fruits and barks contain flavonoids with significant antibacterial activities. The flavonoids isolated using ethyl acetate and ethanol (Table 5) showed zones of inhibition ranging from 0 to 22 mm. It is clear from the results that, *K. africana* fruits and barks contain flavonoids that exhibit antibacterial activities which are comparable to that of mupirocin, a topical antibacterial agent cream used to treat infections on diabetic ulcers. The flavonoid separating at R_f 0.93 showed the best results. Table 6 shows that 99.9% ethanol and water do not exhibit significant antibacterial activity implying that any antibacterial activity observed is due to the extracted flavonoids.

Quantitation of flavonoids

The present study showed the presence of flavonoids in the extracts of *K. africana* as there was an absorbance at 426 nm which was comparable to that of quercetin (Figure 3). The UV-Vis spectrophotometric analyses of flavonoids are based on the formation of complexes between aluminium cations from aluminium chloride and the hydroxyl groups of the flavonoids (Popova et al. 2004). Interferences from other phytochemicals are reduced when complexes with bathochromic effect are formed as a result of the dative bonds formation between aluminium cations and the flavonoids (Silva et al. 2009). The results from Table 3 showed that the ethanol extract contained flavonoids at concentrations of 213.49 ± 0.001 mg/L, whilst the ethyl acetate extract flavonoid concentrations were 36.614 ± 0.001 mg/L. The intensity of the yellow colour

of the complex, flavonoid-Al^{3+} was proportional to the flavonoid concentration in the sample. Quercetin was used as the standard in this study hence the results obtained were total flavonoids expressed per quercetin equivalent.

Conclusion

The findings from the present study show that *K. africana* fruit and bark extracts consist of flavonoids with significant antibacterial and antiradical activities. The flavonoids with greater activity were extracted with ethanol. Thus, the results support the use of *K. africana* fruit and bark powders and water infusions in herbal management of wounds and diabetic ulcers by the local people. The results also show that *K. africana* fruit and bark powder flavonoids are good candidates for polyherbal formulations of topical creams for diabetic ulcers.

Acknowledgements

The authors express their deepest gratitude to the Bindura University of Science Education Research Board for the unwavering support especially providing funds for purchasing chemical reagents.

Conflict of interest

Authors declare no conflict of interest.

References

Atta EM, Mohamed NH and Abdelgawad AA 2017 Antioxidants: an overview on the natural and synthetic types. *Eur. Chem. Bull* 6: 365–375.

Citron DM, Goldstein EJ, Merriam CV, Lipsky BA and Abramson MA 2007 Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. *J. Clin. Microbiol.* 45: 2819–2828.

Dang CN, Prasad YD, Boulton AJ and Jude EB 2003 Methicillin-resistant *Staphylococcus aureus* in the diabetic foot clinic: a worsening problem. *Diabet. Med.* 20: 159–161.

Dennis CN and Wulandari S 2017 Antibacterial effect of ethanol extract of the avocado seed (*Persea americana* Mill.) as an alternative root canal irrigants against *Porphyromonas gingivalis* (in vitro). *Int. J. Appl. Dental Sci.* 3: 89–93.

Doughari JH 2012 Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In *Phytochemicals-A global perspective of their role in nutrition and health*. *InTech* 174.

Dzomba P and Muchanyereyi N 2012 Potential antimicrobial plant extract based therapeutics from *Temnocalyx obovatus* roots. *European J. Med. Plants* 2: 209–215.

Dzomba P, Togarepi E and Musekiwa C, 2012 Phytochemicals, antioxidant and antibacterial properties of a lichen species *Cladonia digitata*. *Afr. J. Biotechnol.* 11: 7995–7999.

Goveas SW and Abraham A 2013 Evaluation of antimicrobial and antioxidant activity of stem and leaf extracts of *Coscinium fenestratum*. *Asian J. Pharm. Clin. Res.* 6: 218–221.

Gu L, Wu T and Wang Z 2009 TLC bioautography-guided isolation of antioxidants from fruit of *Perilla frutescens* var. acuta. *LWT-Food Sci Technol.* 42: 131–136.

Gwatidzo L, Dzomba P and Mangena M 2018 TLC separation and antioxidant activity of flavonoids from *Carissa bispinosa, Ficus sycomorus*, and *Grewia bicolar* fruits. *Nutrire BioMed Central* 43: 1–7.

Ignat I, Volf I and Popa VI 2011 A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chem.* 126: 1821–1835.

Kay CD, Hooper L, Kroon PA, Rimm EB, Cassidy A 2012 Relative impact of flavonoid composition, dose and structure on vascular function: a systematic review of randomised controlled trials of flavonoid rich food products. *Mol. Nutr. Food Res.* 56: 1605-1616.

Khandelwal P, Sharma RA and Agarwal M 2016 Phytochemical analyses of various parts of *Prosopis cineraria. Int. J. Pharm. Chem.* 2: 6–9.

Kumar S and Pandey AK 2013 Chemistry and biological activities of flavonoids: an overview. Hindawi Publishing Corporation *The Scientific World Journal* 2013. http://dx.doi.org/10.1155/2013/162750.

Lipsky BA, Armstrong DG, Citron DM, Tice AD, Morgenstern DE, Abramson MA 2005 Ertapenem versus Piperacillin tazobactam for diabetic foot infections (SIDESTEP): prospective, randomised, controlled, double-blinded, multicentre trial. *Lancet* 366: 1695-1703.

Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJC, Armstrong DG, Deery HG, Embil JM, Joseph WS, Karchmer AW, Pinzur MS, Senneville E 2012 2012 Infectious Diseases Society of America Clinical Practice Guideline for the Diagnosis and Treatment of Diabetic Foot Infections. *Clinical Infectious Diseases* 54(12): 132–173.

Mishra A, Sharma AK, Kumar S, Saxena AK and Pandey AK 2013 *Bauhinia variegata* leaf extracts exhibit considerable antibacterial, antioxidant and anticancer activities. *BioMed Res. Int.* 2013.

Mohammed IS 1996 Phytochemical Studies of Flavonoids from *Polygonum glabrum* L of Sudan. INIS-SD—077. SD9900041.

Mohdaly AAA, Sarhan MA, Smetanska I and Mahmoud A 2009 Antioxidant properties of various solvent extracts of potato peel, sugar beet pulp and sesame cake. *J. Sci. Food Agric.* 90: 218–226.

Muchuweti M, Bhebhe M, Chipurura B, Kasiyamhuru A and Chitindingu K 2013 Determination of profiles, antioxidant activity and quantity of phenolic compounds in Bambara nut (*Vigna subterranea*) varieties found in Zimbabwe. *The African J. Plant Sci. Biotechnol.* 7: 93–98.

Mumin A, Akhter KF, Abedin Z, Hossain Z 2006 Determination of caffeine by solid phase extraction and high performance liquid chromatography (SPE–HPLC). *Malays. J. Chem.* 8: 45–51.

Olalye MT and Rocha JB 2007 Commonly used tropical medicinal plants exhibit distinct in vitro antioxidant activities against hepatotoxins in rat liver. *Exp. Toxicol. Pathol.* 58: 433–438.

Popova M, Bankova V, Butovska D, Petkov V, Damyanova BN, Sabatini AG, Marcazzan GL and Bogdanov S 2004 Validated methods for the quantification of biologically active constituents of poplar-type propolis. *Phytochem. Anal.* 15: 235–240.

Saini S, Kaur H, Verma B, Ripudaman and Singh SK 2009 *Kigelia africana* (Lam.) Benth. — An overview. *Nat. Prod. Rad.* 8: 190–197.

Sen A and Batra A 2012 Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach*. *Int. J. Curr. Pharmaceut* 4: 67–73.

Silva KGH, Xavier J FH, Farias IEG, Silva AKA, Caldas NJA, Souza LCA, Santiago RR, Alexandrino JF, Nagashima JT, Soares LAL, Santos-Magalhães NS and Egito EST 2009. Stationary cuvette: a new approach to obtaining analytical curves by UV-Vis spectrophotometry. *Phytochem Anal.* 20: 265–271.

Stalikas CD 2007 Extraction, separation, and detection methods for phenolic acids and flavonoids. *J. Sep. Sci* 30: 3268–3295.

Uddin B, Nahar T, Khalil MI and Hossain S 2007 In vitro antibacterial activity of the ethanol extracts of *Paederia foetida L*. (Rubiaceae) leaves. *Bangladesh J. Life Sci.* 19 141–143.