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ORIGINAL ARTICLE

New Phytochemical Constituent and Bioactivities of *Horwoodia dicksoniae* and *Rumex cyprius*

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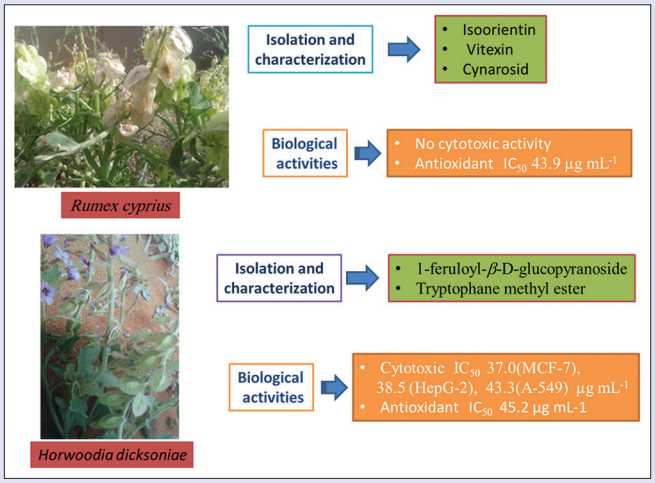
ABSTRACT

Background: Many plants growing in Saudi Arabia are used in folk medicine for treatment of several diseases. **Objective:** Information of the chemical constituents and biological activities of plants is desirable for the discovery of therapeutic agents and discovering the actual value of folkloric remedies. **Materials and Methods:** The compounds were isolated and purified using silica gel column chromatography and preparative high-performance liquid chromatography-diode array detector (HPLC-DAD) Method. The alcoholic extracts of these plants were evaluated for biological activities. **Results:** Isolation and characterization of 1-feruloyl-β-D-glucopyranoside (1) as well as new secondary metabolite tryptophan methyl ester (2) were isolated for the 1st time from the *Horwoodia dicksoniae*. The three flavones were isolated from *Rumex cyprius* identified as isoorientin (3), vitexin (4), and Cynarosid (5). The structures of these compounds were characterized by nuclear magnetic resonance and mass spectrometry analysis and comparing with literature. The compounds were isolated and purified using silica gel column chromatography and preparative HPLC-DAD Method. The alcoholic extracts of these plants were evaluated for antimicrobial activities against two Gram-positive bacteria, two Gram-negative bacteria, and four pathogenic fungi. Both plants showed good activities against *Syncephalastrum racemosum* and *Streptococcus pneumoniae* with minimal inhibitory concentrations (MICs) 0.98 and 1.95 μg/mL, respectively. *H. dicksoniae* showed good activity against *Aspergillus fumigates* with an MIC 1.95 μg/mL. The two extracts showed also effective free radical scavenging activities in the 1,1-diphenyl-2-picrylhydrazyl assay. *H. dicksoniae* exhibited remarkable cytotoxic activity against Human breast cancer mammary cancer cells-7, Human liver cancer human hepatoma carcinoma cells-2, and human lung carcinoma (A-549) cell lines. **Conclusions:** It was suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

Key words: Antimicrobial, antioxidant, cytotoxicity, horwoodia, *Rumex*

SUMMARY

- New secondary metabolite Tryptophane methyl ester as well as 1-feruloyl-β-D-glucopyranoside were isolated for the first time from the HD.
- Isoorientin, vitexin and Cynarosid were isolated from RC.
- HD exhibited good activity against *Aspergillus fumigates* with an MIC 1.95 μg mL^{1}.
- HD showed significant cytotoxic activity against Human breast cancer (MCF-7), Human liver cancer (HepG-2) and Human lung carcinoma (A-549) cell lines.



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INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicine, and chemical precursors for synthetic drugs. Information of the chemical constituents of plants is desirable for the discovery of therapeutic agents and discovering the actual value of folkloric remedies.[1] Many plants growing in Saudi Arabia are used in folk medicine for treatment of several diseases.[2-4] *Horwoodia dicksoniae* Turrill is known in Arabic as Khuzama. *Rumex* genus belongs to polygonaceae family, and several species of this genus have indicated noteworthy therapeutic potentials. There are some reports in literature about evaluation of a few species of *Rumex* for various medicinal potential[5] *Rumex cyprius* revealed antibacterial activity against methicillin resistant *Staphylococcus aureus*, which has shown relatively high resistant when exposed to reference antibiotics.[6] In the present work, phytochemical analyses as well as antimicrobial, antioxidant, and cytotoxic activities of the alcoholic extracts of these plants were carried out.

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MATERIALS AND METHODS

Experimental

General

Ultraviolet (UV) spectra were determined with a Shimadzu UV-1650PC spectrophotometer; infrared (IR) spectra were carried out on a Nicolet 205 Fourier transform IR spectrometer connected to a Hewlett-Packard ColorPro Plotter. ESIMS (positive ion acquisition mode) was carried out on an XEVO TQD triple quadruple instrument (Waters Corporation, Milford, MA 01757, USA) mass spectrometer. Proton nuclear magnetic resonance (^{1}HNMR) and ^{13}CNMR spectra were obtained in dimethyl sulfoxide (DMSO)-d_6 on a Varian Gemini 400 MHz spectrometer using tetramethylsilane as internal standard; chemical shifts are reported as (ppm) at the Department of Chemistry, Faculty of Science, King Abdul-Aziz University, Jeddah 21589, KSA. Preparative high-performance liquid chromatography (HPLC) was performed on a Waters® 2545 Quaternary Gradient Module (150 mm × 19 mm, ID) column, prepacked with Prep C_{18} (OBD) with flow rate 24 mL/min, Waters® fraction collector III and Waters® 2707 auto-sampler. The entire system was controlled using Empower 3 Software. Detection was achieved with a Waters® 2998 diode array detector, and chromatograms were recorded at 254–400 nm. Vacuum liquid chromatography (VLC) was carried out using Silica Gel 60, 0.04–0.063 mm mesh size (Merck). Solid phase extraction (SPE) was performed on SPE-C_{18} cartridges (A Strata column). Thin layer chromatography was carried out on precoated silica gel 60 F254 (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100°C for 5 min.

Plant material

H. dicksoniae and *R. cyprius* herbs were collected from Rafha region, Kingdom of Saudi Arabia, in March, 2014, and were kindly identified by Dr. Abdelrahman Talhah Abdelwahab Abdelrahman, Head of biology department, Faculty of Science, Northern Border University, Kingdom of Saudi Arabia. A voucher specimen has been deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Northern Border University, Kingdom of Saudi Arabia.

Preparation of plant extracts

The air-dried powdered herbs of *H. dicksoniae* (200 g) and *R. cyprius* (250 g) were subjected to exhaustive extraction with 70% methanol (2 L × 3 L) at room temperature. The combined methanolic extracts were concentrated under reduced pressure to dryness which gives 15 and 20 g, respectively. The concentrated methanolic extracts were suspended separately in distilled water (100 mL) and partitioned successively with n-hexane, chloroform, ethyl acetate and *n*-butanol to give *n*-hexane, chloroform, ethyl acetate, *n*-butanol and water fractions for each plant.

Preliminary phytochemical screening

The chemical tests were carried out on the alcoholic extract or the powdered plant material using the procedures outlined by Harborne,[7] Trease and Evans.[8] The results are presented in Table 3.

Isolation of the compounds

The MeOH fraction (15 g) of *H. dicksoniae* was subjected to VLC on silica gel (300 g) using 0.5 L each of n-hexane, n-hexane/EtOAc (50:50) and (25:75), EtOAc, EtOAc/MeOH (1:1), and MeOH to give seven fractions (A1-A7). Fraction F5 (1.2 g) was applied to a sephadex LH-20 column to deliver eight subfractions (1–8). Subfraction 2 and 3 were subjected to subsequent purification on semi-preparative RP-C18 HPLC eluting with MeOH/H_2O (75:25) using XBridge _ RP-18 (150 mm, 10 mm, 5 μ) at a flow rate of 6.6 mL/min and detection at 254 nm to give compounds 1 (30 mg, 20 min) and 2 (20 mg, 33 min), respectively.

Table 1: Nuclear magnetic resonance data of compound 2 (CD_3OD, 400 and 100 MHz)

Number	^{1}H	^{13}C	HMBC
1	-	-	-
2	7.09, s	125.25, CH	
3	-	109.49, C	-
4	7.57, d, 7.4	119.33, CH	C-8, 6
5	6.93, t, 7.3	120.09, CH	C-9, 7
6	7.00, t, 7.7	122.73, CH	C-4, 8
7	7.24, d, 8.2	112.45, CH	C-5, 9
8	-	138.32, C	-
9	-	128.43, C	-
10	3.05, 3.40, brs	28.37, CH_2	
11	3.75, m	52.13, CH	
12	-	170.25, C	-
OCH_3	3.66, s	53.40, CH	C-12

HMBC: Heteronuclear multiple bond correlation

1-Feruloyl-β-D-glucopyranoside (1)

Yellow amorphous powder; λ nm: 237, 329; IR (KBr) ν/cm; ^{1}HNMR (400 MHz, CD_3OD) δ 3.43 (m, 1H, H-4').,45 (m, 1H, H-2'), 3.50 (t, 1H, *J* 8.2, H-3'), 3.55 (m, 1H, H-5'), 3.61 (dd, 1H, *J* 4.7, 11.2, H-6'b), 3.81 (m, 1H, H-6'a), 3.88 (s, OMe), 5.62 (d, 1H, *J* 7.6, H-1'),6.39 (d, 1H, *J* 15.8, α), 6.82, (d, 1H, *J* 8.2, H-5), 7.08 (brd, 1H, *J* 8.1, H-6), 7.17 (d, 1H, *J* 1.3, H-2), 7.71 (d, 1H, *J* 15.8, β), d,; ^{13}CNMR (100 MHz, CD_3OD) δ 55.14, 60.98, 69.72, 72.67, 76.62, 77.37, 94.41,110.49, 113.33, 115.18, 123.04, 126.16,146.95, 147.96, 149.47, 166.43; ESIMS m/z 355 $[M-H]^-$.

Tryptophan methyl ester (2)

Light brown amorphous powder; λ nm: 237, 329; IR (KBr) ν/cm: ^{1}HNMR, ^{13}CNMR [Table 1], ESIMS m/z 241 [M+Na] +, 226 [M-Me+Na].

About 10 g of the MeOH extract of *R. cyprius* were subjected to VLC on silica gel (250 g) using successively *n*-hexane, *n*-hexane/EtOAc (50:50–25:75), EtOAc, EtOAc/MeOH (1:1) and MeOH 1 L each, to give six fractions (F1-F6). Fraction F3 (2.1 g) was subjected to SPE-C_{18} cartridges to deliver eight subfractions (1–8). Subfraction 3 (100 mg) was injected (200 μL) into the reverse phase preparative HPLC-diode array detector (DAD) using a linear gradient elution with a mixture of two solvents. Solvent A consisted of methanol and solvent B consisted of water. The solvent gradient consisted in a series of linear gradients, starting from 5% to 100% of solvent B over 20 min at a flow rate of 24 mL/min and detection at 210–400 nm to give compounds 1 (10 mg), 2 (13 mg) and 3 (10 mg), respectively.

High-performance liquid chromatography-diode array detector characterization of the isolated flavones

Through comparison of different RP-HPLC retention times of compounds subfraction 3 (13.87, 14.82, and 15.94 min respectively) [Figure 1], it was clear that no quite difference in their polarities. The UV spectra of all compounds show the same lambda maxima because the responsible chromophoric group in all compounds is the same one. The importance is the difference in the UV-absorption intensities Figure 2 between the derivatives, where it originated from the difference in the concentration of the samples.

Vitexin (apigenin-8-C-glucoside) (3)

Yellow amorphous powder; λ nm: 268.1, 340.8; IR (KBr) ν/ν/cm: 3430, 1660, 1610, 1595, 1510; ^{1}HNMR (400 MHz, DMSO-d_6) δ 3.41 (m, 1H, H-6"b), 3.30–3.55 (m, 3H, H-3", 4", 5"), 3.76 (d, 1H, *J* 12.0, H-6"a), 4.08 (m, 1H, H-2"),4.72 (d, 1H, *J* 9.2, H-1"), 6.25 (s, 1H, H-3), 6.68 (s, 1H, H-6), 7.92 (d, 2H, *J* 8.0, H-3', 5'), 7.94 (d, 2H, *J* = 8.0 Hz, H-2', 6'), 13.10 (brs, OH); ^{13}CNMR (100 MHz, DMSO-d_6) δ 61.11, 70.3, 70.87, 73.20, 78.32, 81.26, 98.35, 102.26, 103.73, 104.10, 115.9, 121.54, 128.87,

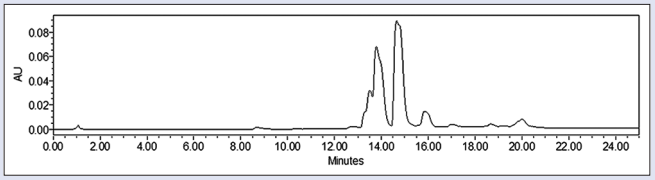

Figure 1: High-performance liquid chromatography-diode array detector chromatogram of subfraction (F-3) of *Rumex cyprius*

155.99, 160.27,160.88, 163.50, 164.16, 182.04; ESIMS m/z 431 $[M-H]^-$, 430 $[M-2H]^-$, 269 [M-H-162] = (aglycone-H).

Isoorientin (luteolin-6-C-glucoside) (4)

Yellow amorphous powder; λ/nm: 270.4, 338.4; IR (KBr) ν/ν/cm: 3400, 1662, 1615, 1590, 1510; ^{1}HNMR (400 MHz, DMSO-d_6) δ 3.19 (1H, m, H-5"), 3.22 (t, 1H, *J* 8.4, H-3"), 3.25 (d, 1H, *J* 8.4, H-4"), 3.45 (d, 1H, *J* 11.6, H-6"b), 3.68 (d, 1H, *J* 11.6, H-6"a), 4.10 (m, 1H, H-2"), 4.59 (d, 1H, *J* 9.6, H-1"), 6.91 (d, 1H, *J* 8.4, H-5'), 6.51 (s, 1H, H-3), 6.64 (s, 1H, H-8), 7.37 (brs, 1H, H-2'), 7.39 (d, 1H, *J* 8.8, H-6'), 13.46 (brs, OH); ^{13}CNMR (100 MHz, DMSO-d_6) δ 61.27, 70.11, 70.31, 72.93, 78.67, 81.17, 93.65, 102.63, 103.24, 108.50, 113.08, 116.08, 119.07, 121.35, 145.58, 149.65, 156.33, 160.43, 163.40, 163.81, 181.8; ESIMS m/z 447 $[M-H]^-$, 493 $[M + HCOOH-H]^-$.

Cynarosid (luteolin-7-O-β-D-glucopyranoside) (5)

Yellow powder; λ nm: 265.7, 342; IR (KBr) ν/ν/cm: 3423, 1637, 1612, 1562, 1459; ^{1}HNMR (400 MHz, DMSO-d_6) δ 3.22 (*t*, 1H, *J* 8.4, H-2"), 3.41 (d, 1H, *J* 11.2, H-6"b), 3.47 (*t*, 1H, *J* 9.2, H-3"),3.47 (*t*, 1H, *J* 10.4, H-4"), 3.73 (d, 1H, *J* 11.2, H-6"b), 3.76 (d, 1H, *J* 12.0, H-6"a), 5.02 (d, 1H, *J* 7.6, H-1"), 6.45 (brs, 1H, H-6), 6.70 (s, 1H, H-3), 6.82 (brs, 1H, H-8), 6.91 (d, *J* 8.4, H-5'), 7.39 (brs, H-2'), 7.44 (d, *J* 8.4, H-6'), 12.77 (brs, OH); ^{13}CNMR (100 MHz, DMSO-*d*6) δ 60.70, 69.41, 72.81, 75.81, 76.71, 94.88, 99.55, 99.60, 102.88, 105.27, 113.19, 119.42, 116.07, 121.26, 145.74, 149.74, 156.90, 160.71, 162.69, 164.64, 181.98; ESIMS m/z 449 $[M + H]^+$, 471 $[M + Na]^+$.

Antimicrobial assays

Antimicrobial activities of plant extracts were investigated *in vitro* against different bacteria and fungi. Two standard strains of Gram-positive bacteria (*Streptococcus pneumonia* Regional Center for Mycology and Biotechnology antimicrobial unit [RCMB 010010], *Bacillus subtilis* [RCMB 010067]) and two standard strains of Gram-negative bacteria (*Pseudomonas aeruginosa* [RCMB 010043], *Escherichia coli* [RCMB 010052]) were used for antibacterial assay. Four clinical pathogenic fungi (*Aspergillus fumigates* [RCMB 02568], *Syncephalastrum racemosum* [RCMB 05922], *Geotricum candidum* [RCMB 05097], and *Candida albicans* [RCMB 05036]) were used for antifungal assay. The microbial species are environmental and clinically pathogenic microorganisms obtained from RCMB, Al-Azhar University. Antimicrobial tests were carried out by the agar well-diffusion method,[9,10] using 100 µL of suspension containing 1×10^8 colony forming units (CFU)/mL for tested bacteria and 1×10^4 spore/mL fungi spread on nutrient agar and malt extract agar, respectively. After the media had cooled and solidified, wells (6 mm in diameter) were made in the solidified agar and loaded with 100 µL of tested sample solutions in 1 mL DMSO with concentrations of 10 mg/mL. Negative controls were prepared using DMSO employed for dissolving the tested samples while ampicillin, gentamycin, and amphotericin B were used as positive controls for Gram-positive bacteria, Gram-negative bacteria, and fungi, respectively. The inoculated plates were then incubated for 24 h at 37°C for bacteria and 48 h at 28°C for fungi and the diameter of any resulting zones of inhibition of growth was measured in millimeter (mm). Each experiment was performed in triplicates and

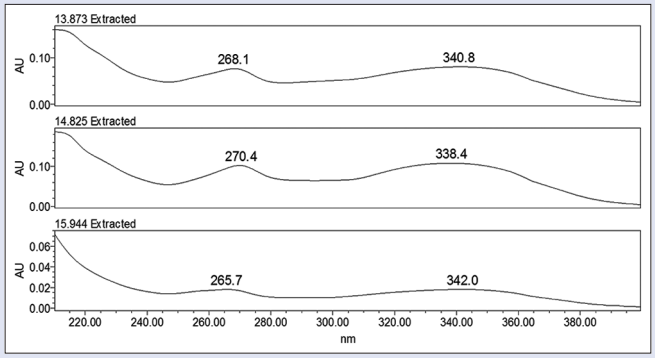

Figure 2: High-performance liquid chromatography-diode array detector chromatogram of compounds 1, 2, and 3, respectively

the data were expressed as mean ± standard deviation. The minimal inhibitory concentrations (MICs) were determined using the two-fold serial dilution technique.[11,12] The two-fold serial dilutions of the tested sample solutions were prepared. The final concentrations of the solutions were 500–0.007 µg/mL. The tubes were then inoculated with the test organisms, grown in their suitable broth for tested pathogenic bacteria (1×10^8 CFU/mL for bacteria and 1×10^4 spore/mL for fungi); each 0.5 mL received 100 µL of the above inoculum and was incubated at 37°C for 24 h for bacteria and after 48 h of incubation at 28°C for fungi. MIC values were taken as the lowest sample concentration that prevents visible bacterial growth. Each experiment was made 3 times.

Antioxidant assay

The antioxidant activity of the plant extracts was determined by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay.[13] Freshly prepared (0.004% w/v) methanol solution of DPPH radical was prepared and stored at 10°C in the dark. A methanol solution of the test sample was prepared. A 40 µL aliquot of the methanol solution was added to 3 mL of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

$$PI = ([(AC - AT)/AC] \times 100)$$

Where AC = absorbance of the control at $t = 0$ min and AT = absorbance of the sample + DPPH at $t = 16$ min.

Cytotoxicity assays

The plant extracts were tested for cytotoxicity against three human tumor cell lines: Human breast cancer mammary cancer cells-7 (MCF-7), Human liver cancer hepatoma carcinoma cells (HepG-2), and Human lung carcinoma (A-549) cell lines. The cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were grown on Roswell Park Memorial Institute 1640 medium (Nissui Pharm. Co., Ltd., Tokyo, Japan) supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were sub cultured 2–3 times a week. The cytotoxic activity was determined using cell viability assay method as described previously.[14,15] Percentage cell viability was calculated as the mean absorbance of control cells/mean absorbance of treated cells. Dose-response curves were prepared and the IC_{50} value was determined. The results are presented in Table 2.

Statistical analysis

All data were expressed as mean ± standard error of the mean. Student's *t*-test was applied for detecting the significance of difference between each sample; $P < 0.05$ was considered statistically significant.[16]

RESULTS AND DISCUSSION

The preliminary phytochemical screening of two medicinal plants, *H. dicksoniae* and *R. cyprius*, is summarized in Table 3. The results revealed the presence of medicinally active compounds in the two plants studied. Flavonoids and tannins were present in both plants. Alkaloids and saponins are present only in *R. cyprius*. Anthraquinones are absent from the two plants. Several studies confirmed that the presence of these important constituents contributes medicinal as well as physiological activities to the studied plants in the treatment of different diseases. Therefore, extracts from these plants can be used as a good source for valuable drugs.

The *H. dicksoniae* and *R. cyprius* extract of was subjected to a succession of chromatographic procedures, including silica gel chromatography, gel permeation chromatography using Sephadex LH-20 and preparative HPLC to afford two pure isolates 1-feruloyl-β-D-glucopyranoside (1), tryptophan methyl ester (2), isoorientin (3), vitexin (4), and cynarosid (5) [Figure 3]. The structures of the isolated compounds were established using spectroscopic analysis, especially, NMR spectra and direct comparison with published data.[17,18]

The antimicrobial activities of the plant extracts were investigated *in vitro* against different bacteria and fungi. *H. dicksoniae* and *R. cyprius* extracts demonstrated growth inhibitory effect and variable antimicrobial activity against most of the specific organisms tested [Table 4 and Figure 4]. They demonstrate a good activity against *Aspergillus fumigatus*, *Streptococcus pneumoniae* and *E. coli*. They appear to be more active against *S. racemosum* than the standard. They showed week antimicrobial activity against *Geotricum candidum* and *B. subtilis*. Both plant extracts did not show antimicrobial activity against the Gram-negative bacteria, *P. aeruginosa* and the fungus *C. albicans*.

The results of the MIC determinations [Table 5 and Figure 5] showed noticeable MIC values for the tested plant extracts against the entire set of the tested organisms except for *P. aeruginosa* and *C. albicans*. All the tested plant extracts exhibited both antibacterial and antifungal activities. The

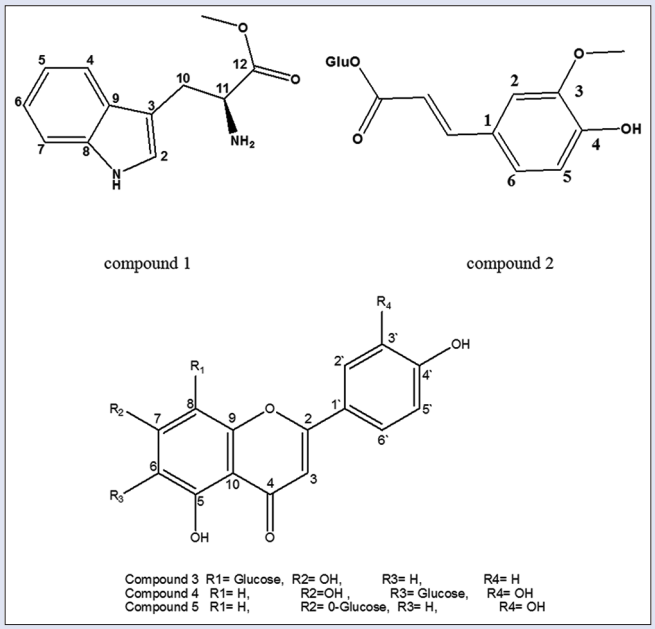

Figure 3: The structures of compounds 1, 2, 3, 4, and 5

Table 2: 1,1-diphenyl-2-picrylhydrazyl scavenging/(half maximal inhibitory concentration) (μg/mL) and cytotoxic activity/(half maximal inhibitory concentration) (μg/mL) of *Horwoodia dicksoniae* and *Rumex cyprius* extracts

Sample	DPPH scavenging/ (μg/mL)	Cytotoxic activity/(μg/mL)		
		HCT-116	HepG-2	A-549
Horwoodia dicksoniae	45.2±1.4	38.5±0.69	37.0±0.16	43.3±0.37
Rumex cyprius	43.9±0.7	>100	>100	>100
Ascorpic acid	14.2±0.6	NA	NA	NA
Vinblastin	NA	2.38±0.17	4.6±0.17	

NA: No activity; IC_{50}: The half maximal inhibitory concentration; DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 3: Phytochemical screening of the *Horwoodia dicksoniae* and *Rumex cyprius*

	Horwoodia dicksoniae	*Rumex cyprius*
Flavonoids	+++	+++
Alkaloids	–	++
Tannins	+++	+++
Saponins	–	+++
Anthraquinones	–	–

–: Equal to negative result; +: Equal to positive result

Table 4: Antimicrobial activity as diameter of inhibition zone/mm of plant extracts against selected microorganisms

Tested microorganisms	Diameter of inhibition zone/mm		
	Horwoodia dicksoniae	*Rumex cyprius*	Standard
Fungi			Amphotericin B
Aspergillus fumigatus (RCMB 02568)	21.2±0.63	20.3±0.25	23.7±0.1
Syncephalastrum racemosum (RCMB 05922)	22.3±0.44	21.6±0.34	19.7±0.2
Geotrichum candidum (RCMB 05097)	24.1±0.25	23.4±0.58	28.7±0.2
Candida albicans (RCMB 05036)	NA	NA	25.4±0.1
Gram-positive bacteria			Ampicillin
Streptococcus pneumoniae (RCMB 010010)	20.6±0.63	20.6±0.44	23.8±0.2
Bacillus subtilis (RCMB 010067)	22.4±0.44	23.4±0.67	32.4±0.3
Gram-negative bacteria			Gentamicin
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	17.3±0.1
Escherichia coli (RCMB 010052)	20.1±0.58	18.6±0.46	19.9±0.3

Data are expressed in the form of mean±SD. RCMB: Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms; NA: No activity

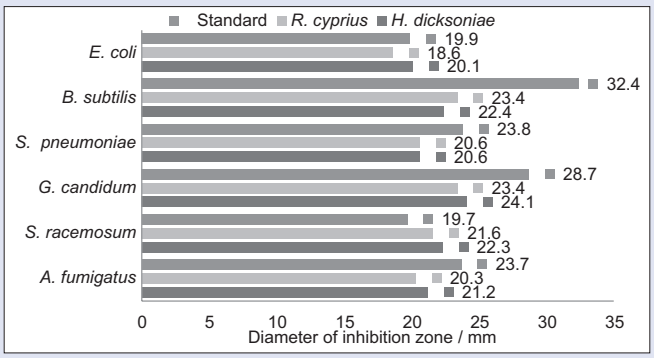

Figure 4: Antimicrobial activity as diameter of inhibition zone/mm of plant extracts

obtained MICs varied from 0.98 to 3.9 µg/mL for the tested plant extracts. The lowest MIC values (0.98 µg/mL) observed with the plant extracts against *S. racemosum*, *G. candidum* and *B. subtilis*. When regarding the activity of the positive standard against the tested microbial species, the two plant extracts showed the highest level of activity against *S. racemosum* with an MIC 0.98 µg/mL (nearly fourfold lower than that of amphotericin B), followed by *H. dicksoniae* extract against the fungus *A. fumigatus* and the two plant extracts against the Gram-positive bacteria *S. pneumoniae* with MICs 1.95 µg/mL (nearly only twofold greater than that of amphotericin B and ampicillin). *H. dicksoniae* extract is active against *E. coli* as the same as the standard gentamycin with an MIC 3.9 µg/mL. *R. cyprius* extract showed the lowest level of activity against *A. fumigatus* and *E. coli*.

The antioxidant activity

There are significant variations in the capacity of the plant extracts to scavenge the DPPH radical with IC_{50} ranging from 43 to 45 µg/mL [Table 2, Figures 6 and 7]. From the estimated IC_{50} values, the order of potency is *R. cyprius* extract with IC_{50} 43.9 µg/mL followed by *H. dicksoniae* extract with IC_{50} 45.2 µg/mL. The IC_{50} of these extracts are higher than the IC_{50} of the positive control (ascorbic acid 14.2 µg/mL).

The cytotoxic activity

Cytotoxic activities of the *H. dicksoniae* and *R. cyprius* extracts were tested against three cancer cell lines Human breast cancer (MCF-7), Human liver cancer (HepG-2), and Human lung carcinoma (A-549) [Table 2, Figures 8 and 9]. As a result, *H. dicksoniae* exhibited a remarkable cytotoxic activity against the three cell lines with values of IC_{50} 37.0, 38.5, and 43.3 µg/mL, respectively. On the other hand,

Table 5: Antimicrobial activity as minimum inhibitory concentration/(µg/mL) of plant extracts

Tested microorganisms	Minimum inhibitory concentration/ (µg/mL)		
	Horwoodia dicksoniae	*Rumex cyprius*	Standard
Fungi			Amphotericin B
Aspergillus fumigatus (RCMB 02568)	1.95	3.9	0.98
Syncephalastrum racemosum (RCMB 05922)	0.98	0.98	3.9
Geotrichum candidum (RCMB 05097)	0.98	0.98	0.24
Candida albicans (RCMB 05036)	NA	NA	0.49
Gram-positive bacteria			Ampicillin
Streptococcus pneumoniae (RCMB 010010)	1.95	1.95	0.98
Bacillus subtilis (RCMB 010067)	0.98	0.98	0.24
Gram-negative bacteria			Gentamicin
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	31.25
Escherichia coli (RCMB 010052)	3.9	7.81	3.9

RCMB: Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms; NA: No activity

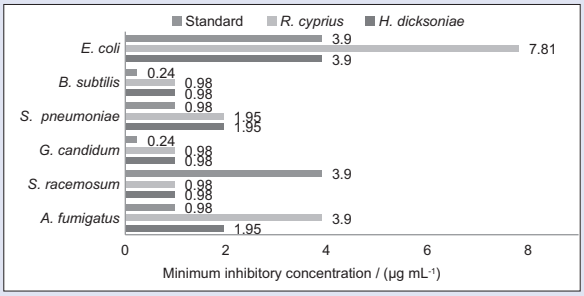

Figure 5: Minimal inhibitory concentrations (µg/mL) of plant extracts

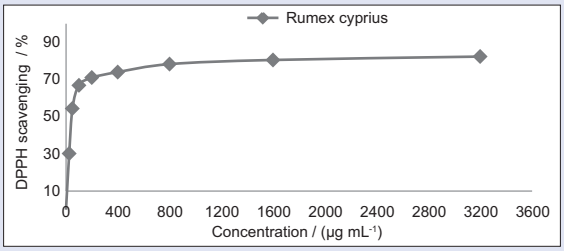

Figure 6: Antioxidant activity of *Rumex cyprius* extract using 1, 1-diphenyl-2-picrylhydrazyl scavenging

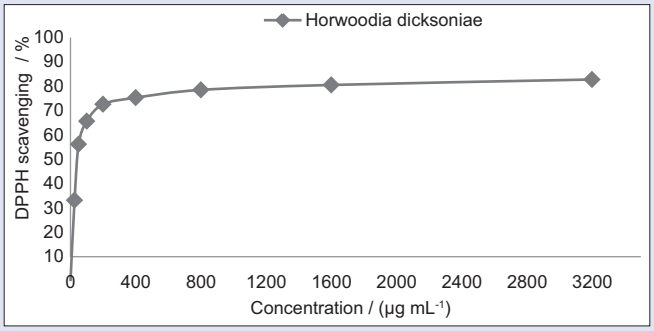

Figure 7: Antioxidant activity of *Horwoodia dicksoniae* extract using 1, 1-diphenyl-2-picrylhydrazyl scavenging

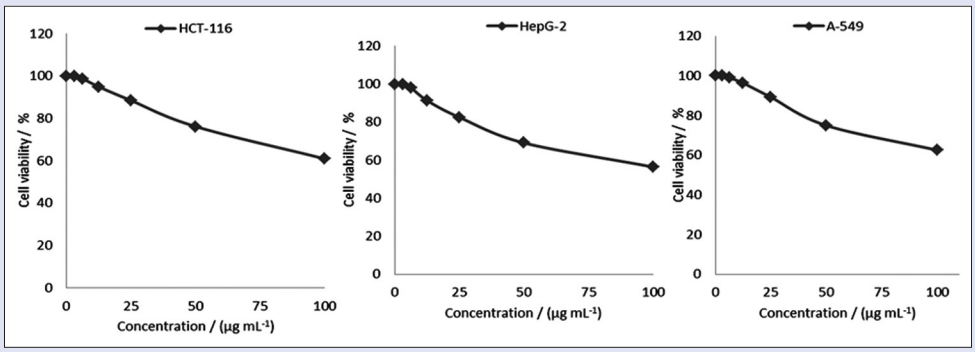

Figure 8: Cytotoxic activity of *Horwoodia dicksoniae* extract against cancer cell lines HCT-116, hepatoma carcinoma cells-2 and A-549 respectively

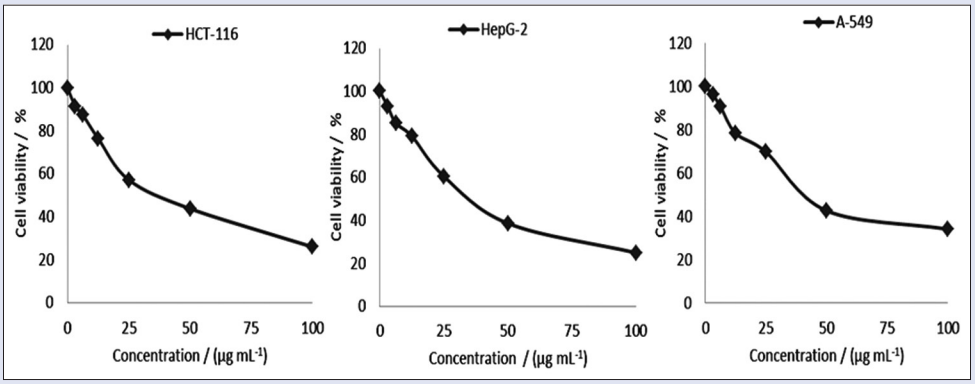

Figure 9: Cytotoxic activity of *Rumex cyprius* extract against cancer cell lines HCT-116, hepatoma carcinoma cells-2 and A-549 respectively

R. cyprius extract showed no cytotoxic activity against the tested cell lines.

CONCLUSIONS

It was suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Also, additional work is encouraged to elucidate the possible mechanism of action of these constituents.

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Conflicts of interest

There are no conflicts of interest.

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