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Elemental, phytochemical, and toxicological assessment of *Cissus rotundifolia* (Forssk.) Vahl

Cissus rotundifolia (Forssk.) Vahl. (Vitaceae) is a wild plant that is commonly used by communities from rural areas as a food and medicine. There are limited studies on the phytochemical composition and the impact of soil quality on the elemental distribution in this plant. In this study, we report a phytochemical analysis to identify the phytocompounds responsible for the reported biological activities of *C. rotundifolia*. We also examined the impact of soil quality on elemental uptake by the edible parts of *C. rotundifolia* collected from eight geographical locations in KwaZulu-Natal (South Africa) to assess the nutritional benefits and potential heavy metal toxicities. Three secondary metabolites (stigmasterol, β-sitosterol, and pheophytin *a*) were isolated, and their structures were characterised by high-resolution mass spectrometry and nuclear magnetic resonance data. The plant was found to contribute adequately to the recommended dietary allowances for essential nutrients without exceeding tolerable upper intake limits and with low concentrations of toxic heavy metals. The average concentrations of microelements in the edible parts were found to be in decreasing order of Fe>Mn>Se>Zn>Cu>Cr>Ni>Co. The bioaccumulation factors indicate that the plant controls the uptake of metals from the soil and would make a good indicator and biological monitor for cadmium toxicity. However, a health risk assessment exposed carcinogenic risks on regular consumption of the plant obtained from sites close to pollution sources, such as roads and landfills. The findings from this study show the synergies when consuming medicinal plants and provide evidence for *C. rotundifolia* as a nutraceutical.

Significance:

This study provides additional scientific knowledge on the phytochemical composition of *C. rotundifolia*. Three phytocompounds (stigmasterol, β-sitosterol, and pheophytin *a*) were isolated, and their presence may be correlated to this plant's antidiabetic, anti-inflammatory, and antibacterial properties. This study shows that *C. rotundifolia* contributes adequately to the recommended dietary allowances for essential elements, and the plant is safe for human consumption if collected from non-polluted sites. The carcinogenic and non-carcinogenic estimates for the toxic metals due to consumption of the plant signify the possibility of developing cancer over time if the plant is consumed frequently from polluted sites.

Introduction

The World Health Organization (WHO) estimated that 80% of the world's population depends on plants for their primary needs, such as food and medicine.[1] Medicinal plants are a rich source of bioactive phytochemicals, which can be used directly as new drugs or developed into lead compounds for optimisation into novel drugs against various diseases.[1] Nonetheless, information on most medicinal plants' phytochemistry still needs enhancement.[1] In addition, local people from disadvantaged communities consume wild-growing edible plants to meet their nutritional needs and to diversify their diet.[2,3] Essential elements obtained from these plants are necessary for the metabolic processes and functioning of the human body, and their deficiencies or excessiveness can have detrimental health effects.[4,5] Therefore, it is essential to study the nutritional value of wild edible plants.

Wild edible plants and medicinal plants are often considered safe for human consumption; however, many studies have reported on the contamination of these plants by toxic elements from their growing environments.[6-8] As a result, studies focused on evaluating edible plants for heavy metal toxicity are of great importance to human health. *Cissus rotundifolia* (Forssk.) Vahl. (Vitaceae) is a wild, edible medicinal plant that grows in various regions of East and Central Africa, Zimbabwe, Mozambique, Egypt, Yemen, Saudi Arabia, and South Africa.[9,10] The plant has several biological activities, including antioxidant, antibacterial, anti-diabetic, anti-inflammatory, anti-fertility, anti-ulcerative, anti-parasitic, antimalarial, and cytotoxicity.[9,11-13]

Previous phytochemical studies on *C. rotundifolia* resulted in the characterisation of a triterpene, flavonoids, steroidal saponin, cissuxinoside, and cissoic acid.[9,14] Another study on the phytochemistry of *C. rotundifolia* identified 1-O-(4-coumaroyl)-β-D-glucopyranose as an active principle against diabetes.[9] However, compounds responsible for the other biological activities of this plant have not been identified. The cooked leaves of *C. rotundifolia* are widely consumed by people in rural areas of South Africa and other parts of the world for their nutritional and therapeutic benefits.[9,15] The plant is also used as a cheap thickening agent.[10] The leaves and fruits of *C. rotundifolia* collected from different regions of Saudi Arabia, and Yemen were reported to contain enough essential nutrients, including carbohydrates, proteins, fats, vitamins, minerals, and amino acids.[15-18] The mineral composition of the stem of *C. rotundifolia* and the impact of soil quality on elemental distribution in this plant have not been reported.

In this study, we report on the phytochemical analysis of *C. rotundifolia*, intending to find additional bioactive compounds responsible for the plant's purported biological activities. We also report on the elemental composition of the leaves and stems collected from different geographical sites in KwaZulu-Natal (South Africa) to assess the plant's nutritional benefits and potential heavy metal toxicities.

Materials and methods

Sample preparation and analysis

C. rotundifolia plant and soil samples were collected from eight sites in KwaZulu-Natal, South Africa (Figure 1): Margate (S1), Ballito (S2), Richards Bay (S3), Shongweni (S4), Cato Ridge (S5), St Lucia (S6), Amanzimtoti (S7), and Umgababa (S8). Except for sampling sites S4 and S5, the sites are along the coast; it was assumed that they are non-polluted as they are far from roads, landfills, and other pollution sources. S4 and S5 are inland and very close to pollution sources (S4 is close to a landfill site, and S5 is close to a railway line and a truck stop).

Collected plant material was placed in plastic bags, sealed, and stored. Plant specimens from each sampling site were submitted for authentication by the taxonomist, Mr Edward Khathi, in the School of Life Sciences (University of KwaZulu-Natal, South Africa), where a voucher specimen (Buthelezi C 01-18274) was deposited. The soil was collected from the eight sites at 10–15 cm below the plant's roots using a plastic spade. Plant samples were washed with double-distilled water, dried in the oven at 50 °C for 24 h, and then crushed using a pestle and mortar. Plant material from site 7 (Amanzimtoti) was used for phytochemical analysis due to the large quantity available at this site. Composite soil samples from each site were obtained by coning and quartering. Soil samples (10 g) were sieved through a 2 mm mesh sieve to get uniform particle size, air dried, and crushed using a mortar and pestle. All dried samples were stored in sealed plastic bags and kept in a refrigerator at 4 °C.

Nuclear magnetic resonance (NMR) analysis was performed using the Bruker Avance III spectrometers (400 MHz and 600 MHz). The isolated compounds were dissolved in deuterated chloroform ($CDCl_3$) or methanol (MeOD), and tetramethylsilane was used as the internal standard. Chemical shifts (δ) and coupling constants are reported in ppm and Hertz (Hz), respectively. Column chromatography was packed using Merck silica gel 75–230 μm. Thin-layer chromatography (TLC) plates were pre-coated with Merck silica gel 60 F254. Agilent GC-MSD was used to obtain the mass of the compounds.

The elemental analysis of the plant and soil was performed using analytical grade solvents from Sigma Aldrich (St. Louis, MO, USA). The laboratory glassware and the glass bottles were soaked in 3 M nitric acid (HNO_3), rinsed with double distilled water, and air dried. A CEM Microwave Accelerated Reaction System with MARSXpress™ vessels and infrared temperature sensors (CEM Corporation, NC, USA) was used for acid digestion of the plant samples. Inductively coupled plasma-optical emission spectrometry (ICP-OES) (PerkinElmer, Optima 5300Dual View, Billerica, MA, USA) was used for elemental analysis.

Extraction and purification

The dried powdered roots (572 g), stems (461 g), and leaves (562 g) were extracted with dichloromethane (DCM) and MeOH for 48 h consecutively. The extracts were filtered and concentrated using a rotary evaporator to give the crude extracts, which were stored in the fridge until further use. The extracts from the stems and leaves had similar TLC profiles. The DCM crude extract from the roots (11.65 g), stems (17.65 g), and leaves (50.44 g) were fractioned on a silica gel column using different proportions of hexane and ethyl acetate (starting from 100% hexane and increasing the polarity by 10% to 100% ethyl acetate). The collected fractions were profiled using TLC to give six main fractions. Compound **1** (110 mg, 0.94% of the DCM extract) and compound **2** (35 mg, 0.3% of the DCM extract) were isolated from fractions 1 and 2 of the DCM root extract, respectively. The DCM extract of the leaves was fractionated to give three main fractions, and compound **3** (6.4 mg, 0.013% of the DCM extract) was isolated from fraction 2.

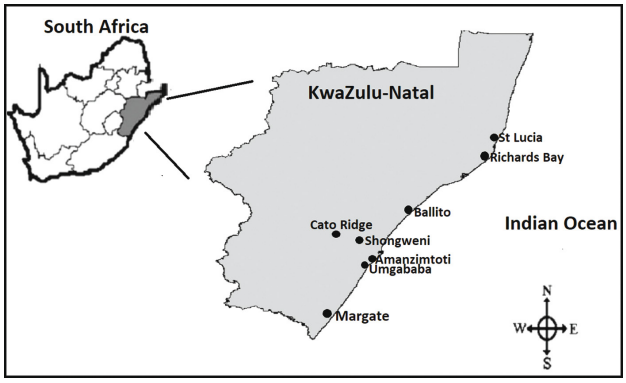

Figure 1: Map showing selected sampling sites in KwaZulu-Natal, South Africa.

Elemental analysis

Plant material (0.25 g) and the certified reference material (CRM) were weighed into microwave vessels with 10 mL HNO_3 in three replicates. The samples were pre-digested for 30 min before microwave digestion. The power was set to 100% (1600 W at 180 °C) for 20 min. The holding time and cooling times were set to 15 min each. After digestion, the samples were filtered through 0.45 μm nylon membrane filters with a 25 mm syringe into 50 mL volumetric flasks topped up to the mark with double-distilled water, transferred into 15 mL polypropylene vials, and refrigerated at 4 °C before analysis. The elements measured were: arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), palladium (Pd), selenium (Se), and zinc (Zn); the elements were measured using ICP-OES and concentrations are presented as mg/kg dry weight (DW) of the sample.

Quality assurance

The validity of an analytical method was determined by evaluating the certified reference material (CRM, strawberry leaves, LGC-7162, Community Reference Bureau of the Commission of the European Communities, Brussels, Belgium). The wavelength which produced the most acceptable CRM results with the highest intensity and no interfering elements (Table 1) was selected. Reagent blanks and calibration standards for each element in double-distilled water (within the estimated ranges) that were prepared from 1000 mg/L stock standard solutions (Fluka Analytical, Sigma, Switzerland) were used to produce five-point calibration curves relating to concentration strength. The best linear fit of the curves was chosen. All samples, including calibration standards and blanks, were analysed in 70% nitric acid to eliminate matrix effects and reduce spectral interferences. The CRMs were analysed to verify the correctness and exactness of the calibration curve and were also used to accept or reject the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) were also determined to validate the method.

The method's accuracy was determined by comparing mean experimental values from three replicates to the certified values for an analyte using the CRMs (Table 1). A two-sample *t*-test (assuming equal variances) confirmed the accuracy as there were no statistically significant differences between the means ($p>0.05$). Method precision, which shows measurement closeness after repeated analysis of an analyte, was estimated by comparing the %RSD for the CRM. This should be within 20% of the true value. The experimental values at the 95% confidence interval were within the appropriate range of that stipulated for the CRM. Therefore, the analytical method was accepted.

Bioaccumulation factor and pollution indicator

The bioaccumulation factor (BAF) defines the ability of plants to accumulate and store heavy metals in the soil. BAF is calculated as the plant's metal concentration ratio to the metal concentration in the corresponding soil (Equation 1).[19]

$$BAF = Metal\frac{plant}{Metal}soil \qquad \text{Equation 1}$$

The geoaccumulation index (I_{geo}) determines the soil's degree of metal pollution in the seven grades ranging from uncontaminated to extremely contaminated (Equation 2).[20]

$$Igeo = log2\left[\frac{Cn}{1.5Bn}\right], \qquad \text{Equation 2}$$

Table 1: The detection limit (DL) of the instrument, limit of quantitation (LOQ), certified and measured values for the certified reference material (CRM – LGC7162 strawberry leaves and soil D081-540, $n = 3$), based on dry mass

Elements	Wavelength (nm)	DL (mg/kg)	LOQ	Plant CRM	Measured	Soil CRM	Measured	Acceptable
As	193.696	0.0100	0.0303	–	–	88.4	120.7±14.42	72–105
Cd	228.802	0.0027	0.0082	–	–	143	154.9±13.6	116–169
Co	228.616	0.007	0.0212	–	–	199	229±52.3	166–233
Cr	267.716	0.0071	0.024	2.15±0.34	2.02±0.04	86.8	88.67±14.81	69.3–104
Cu	324.752	0.0054	0.0164	10	10.35±0.14	268	279±55.1	219–317
Fe	238.204	0.0062	0.0188	818±48	820.79±7.82	12 800	12 170±5388	5380–20 100
Mn	257.61	0.0016	0.0048	171±10	180.19±0.84	425	521±114.5	347–502
Ni	231.604	0.0480	0.1455	2.6± 0.7	2.72±0.06	236	255± 26.8	194–279
Pb	220.353	0.09	0.2727	1.8±0.4	1.78±0.10	97.9	137.52±30.6	80–116
Zn	213.857	0.0018	0.0055	24±5	25.26±0.48	130	143.9±38.1	106–115

where Cn is the elemental concentration, Bn is the total baseline concentration, and 1.5 is used to reduce background value variations to the rock composition variations.

Health risk assessment

The estimated daily intake (EDI) of the toxic metals was measured in mg/kg body weight per day[21]:

$$EDI = [X]x\ IR/Bw, \quad \text{Equation 3}$$

where [X] is the concentration of the toxic metal (mg/kg, DW), IR is the ingestion rate of leafy vegetables per person estimated to be 0.062 kg per day, and Bw is the average human body weight in South Africa, usually 70 kg.[22]

The target hazard quotient (THQ) is the ratio of exposure to a toxic metal with its reference dose (Equation 4), and it estimates the non-carcinogenic risk caused by exposure to the toxic metal.[23,24] For each toxic metal, a THQ<1 indicates low risk, and THQ≥1 shows high risk. RfD_0 is the safe oral reference dose of elements (mg/kg/day) calculated for adults. The RfD_0 values are as follows: As (0.0003), Cd (0.001), and Pb (0.004).

$$THQ = EDI/RfDo \quad \text{Equation 4}$$

Carcinogenic risk (CR) estimates the probability of developing cancer due to exposure to toxic metals over a lifetime (Equation 5).[22,23]

$$CR = EDI\ x\ CPSo, \quad \text{Equation 5}$$

where CPS_0 is the oral slope factor of the toxic metal (mg/kg/body weight/day). The CPS_0 values are as follows: As (1.5), Cd (6.3), and Pb (0.0085).[24] For each toxic heavy metal, a $CR > 1 \times 10^{-4}$ indicates a high probability of carcinogenic risk.[23]

Statistical analysis

A comparative study and grouping of results were performed using a one-way analysis of variance (ANOVA) and Tukey's post-hoc test to determine and evaluate significant differences between means. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) (PASW version 24, IBM Corporation, Cornell, NY, USA).

Results and discussion

Phytochemical investigation

Two phytosterols (stigmasterol and β-sitosterol) and pheophytin *a* were isolated from the leaves and the roots of *C. rotundifolia*. The compounds (Figure 2) were identified using spectroscopic data (NMR, GC-MS/LC-MS, IR, and UV/Vis) and by comparison of the experimental data with values in the literature. Compound **1** (110 mg), a white crystalline powder from the DCM extract of the roots with molecular ion peak at *m/z* 412 [M^+] corresponding to $C_{29}H_{48}O$, was identified as stigmasterol.[25] Stigmasterol is commonly isolated from the genus *Cissus*.[25-28] However, this is the first report on its isolation from *C. rotundifolia*. This compound is known for its antibacterial, antioxidant, anticancer, and cholesterol-lowering properties, which may contribute to the healing properties of *C. rotundifolia*.[29,30] A white powder (35 mg) (compound **2**) with a molecular ion peak at *m/z* 414 corresponding to $C_{29}H_{50}O$ was obtained from the root of *C. rotundifolia*. The ^1H and ^{13}C NMR spectral data confirmed the compound to be β-sitosterol, previously isolated from *C. rotundifolia* and other plants in the genus, such as *C. quadrangularis* and *C. sicyoides*.[25,26] β-sitosterol is known for its antibacterial activity, which may also justify the use of *C. rotundifolia* in wound healing.[31] Compound **3**, isolated as a dark green solid (120 mg) from the DCM extract of the leaves, with molecular ion peak at *m/z* 871 [M^+] corresponding to $C_5H_{74}N_4O_5$, was identified as pheophytin *a*.[32] This is the first isolation of pheophytin *a* from *C. rotundifolia*, but this compound was isolated from *C. assamica* and *C. quadrangularis* in the genus *Cissus*.[33,34] Pheophytin *a* may contribute towards the antioxidant and anti-inflammatory properties of the crude extract of *C. rotundifolia*.[32,34]

Elemental composition

We evaluated the concentrations of 11 metals in *C. rotundifolia* (leaves and stems) and the corresponding soil samples collected from eight different sampling sites in KwaZulu-Natal (Figure 1). Except for sites 4

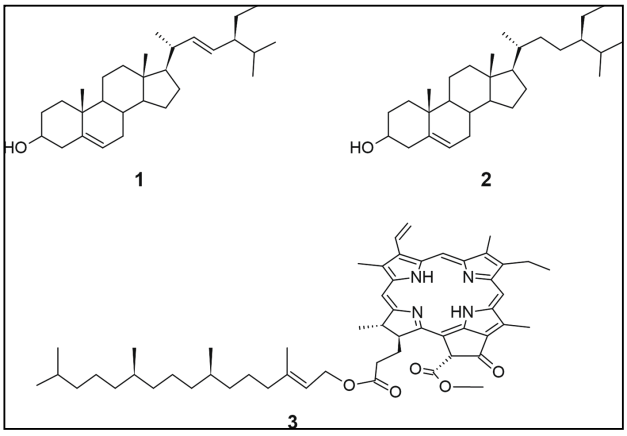

Figure 2: Compounds isolated from *Cissus rotundifolia.*

(Richards Bay) and 5 (Cato Ridge), higher concentrations of Mn were detected in the plant than in the soil (Table 2, BAF>1). This could mean that the plant can take up the required concentrations from the soil to meet its metabolic needs. Alternatively, higher concentrations in aerial plant parts could be due to atmospheric deposition from contaminated sites. *C. rotundifolia* leaves from a mountain in Yemen had an average Mn concentration of 18.9 mg/kg, which is relatively low compared with that in our study (35.1—74.7 mg/kg).[17] However, mountainous regions are considered pristine due to their distance away from anthropogenic sources, which could explain the disparity with our concentrations. A study of the impact of atmospheric deposition on particulate Mn distribution in northwestern Mediterranean surface water[35] showed variations between atmospheric-depositional and settling fluxes based on atmospheric input from high/low productivity seasons. This was also related to more suspended particulate Mn and particulate residence times in standing crops.[35] This study showed that higher concentrations in aerial plant parts could be due to atmospheric deposition, as seen in our study. Another study investigated Mn migration from soil to Scots pine (*Pinus sylvestris* L.) shoots and needles contamination by a steel plant's emissions.[36] Control and contaminated soil concentrations were comparable, but needles at contaminated sites had concentrations of Mn that were 2.5 times higher than those at control sites. This could indicate an increase in metal mobility at contaminated areas with a shift in migration pattern towards Mn accumulation in the aerial parts of the plant, as seen in our study.

For Zn, plant concentrations were higher than soil concentrations at all sites (Table 2). *C. rotundifolia* leaves from a mountain in Yemen[17] had an average Zn concentration of 2.2 mg/kg, and fruits from Shada Mountain in southwest Saudi Arabia[18] had an average Zn concentration of 9.2 mg/kg – these concentrations are relatively low compared to those in our study (19.9—73.1 mg/kg). A study focusing on atmospheric deposition of Zn to forest vegetation in the Tennessee Valley showed

Table 2: Concentration of microelements in *Cissus rotundifolia* (CR) leaves (L), stems (S), and soil from the eight sites (mean (SD), *n*=3), and bioaccumulation factors

Site	Concentration (mg/kg)			Bioaccumulation factor	
	CR Leaves	CR Stems	Soil	[CRL]/[soil]	[CRS]/[soil]
Co					
S1	0.22 (0.18)	0.21 (0.20)	0.1 (0.26)	2.4	2.4
S2	0.03 (0.30)	1.11 (1.67)	0.6 (0.20)	0.1	1.8
S3	0.07 (0.51)	ND	0.6 (0.39)	0.1	ND
S4	ND	0.44 (0.25)	0.7 (0.35)	ND	0.6
S5	ND	0.20 (0.05)	1.1 (0.41)	ND	0.2
S6	0.48 (0.21)	0.41 (0.02)	0.6 (0.65)	0.8	0.7
S7	0.23 (0.02)	0.11 (0.02)	0.3 (0.19)	0.9	0.4
S8	0.01 (0.04)	0.10 (0.04)	0.5 (0.09)	0.0	0.2
Cr					
S1	0.17 (1.05)	1.66 (0.96)	3.0 (0.6)	0.1	0.6
S2	0.82 (0.27)	ND	4.0 (3.4)	0.2	ND
S3	1.01 (0.37)	ND	6.6 (2.94)	0.2	ND
S4	ND	ND	9.7 (0.50)	0.0	ND
S5	ND	ND	9.0 (4.35)	ND	ND
S6	3.42 (0.88)	0.24 (0.36)	4.2 (1.75)	0.8	ND
S7	ND	ND	2.3 (0.75)	ND	ND
S8	ND	ND	6.2 (1.05)	ND	ND
Cu					
S1	10.34 (1.49)	5.9 (0.70)	3.6 (0.33)	2.9	1.7
S2	5.93 (0.08)	4.66 (0.01)	1.8 (0.98)	3.4	2.6
S3	4.29 (0.29)	8.91 (0.07)	3.1 (0.92)	1.4	2.9
S4	4.8 (0.74)	9.2 (1.25)	13.6 (2.45)	0.4	0.7
S5	4.1 (0.92)	8.48 (0.45)	14.3 (0.04)	0.3	0.6
S6	5.52 (0.48)	5.13 (1.09)	1.6 (0.04)	3.4	3.2
S7	5.35 (0.17)	1.96 (0.26)	0.9 (0.31)	5.9	2.2
S8	5.15 (2.1)	3.91 (0.48)	1.8 (0.55)	2.8	2.1

Site	Concentration (mg/kg)			Bioaccumulation factor	
	CR Leaves	CR Stems	Soil	[CRL]/[soil]	[CRS]/[soil]
Fe					
S1	421.5 (86.6)	1565 (431)	1560 (197.9)	0.3	1.0
S2	309.5 (17.7)	119.5 (17.7)	2595 (1619)	0.1	0.0
S3	635.5 (177)	227.5 (13.4)	3320 (876.5)	0.2	0.1
S4	379 (52.4)	118.32 (60.4)	3990 (2022)	0.1	0.0
S5	253.5 (33.2)	124.1 (39.5)	5935 (3288)	0.0	0.0
S6	1032 (53.8)	358.5 (0.71)	2410 (1018)	0.4	0.1
S7	99.8 (10.2)	116.9 (48.2)	1505 (304)	0.1	0.1
S8	178.5 (14.9)	77.35 (13.9)	2855 (261.7)	0.1	0.0
Mn					
S1	74.68 (0.61)	48.97 (2.94)	36 (19.1)	2.1	1.4
S2	51.32 (3.85)	61.43 (0.52)	33 (0.12)	1.5	1.8
S3	49.59 (4.22)	85.95 (8.6)	41 (10.7)	1.2	2.1
S4	35.1 (3.8)	39.55 (10.1)	65 (2.02)	0.5	0.6
S5	48.18 (13.7)	37.97 (2.6)	71 (15.4)	0.7	0.5
S6	65.12 (3.92)	102.4 (6.4)	41 (1.84)	1.6	2.5
S7	44.56 (3.35)	22.24 (3.67)	23 (2.18)	1.9	1.0
S8	36.155 (18.8)	43.11 (4.56)	38 (0.52)	1.0	1.1
Zn					
S1	73.81 (0.26)	35.44 (1.22)	32 (10.9)	2.3	1.1
S2	32.5 (17.8)	42.17 (3.35)	11 (2.56)	2.9	3.8
S3	39.23 (8.15)	37.53 (2.2)	23 (3.7)	1.7	1.6
S4	23.64 (5.75)	38.22 (14.9)	25 (4.1)	1.0	1.5
S5	28.96 (10.7)	52.48 (1.09)	29 (0.93)	1.0	1.8
S6	19.87 (0.19)	31.12 (1.98)	17 (6.5)	1.2	1.8
S7	34.12 (21.2)	23.79 (4.73)	11 (0.39)	3.2	2.2
S8	32.12 (15.6)	23.57 (2.95)	21.1 (1.75)	1.6	1.1

S1, Margate; S2, Ballito; S3, Richards Bay; S4, Shongweni; S5, Cato Ridge; S6, St Lucia; S7, Amanzimtoti; S8, Umgababa

that atmospheric deposition during the growing season contributed to relatively high concentrations due to metal particles deposited on dry leaf surfaces.[37] Another study showed physiological control of trace metal uptake with plant root affinity for Zn declining with increased Zn concentration in the soil solution.[38] High Zn concentrations in our study, similar to Mn, could be due to atmospheric deposition or enhanced uptake mechanisms at the root zone to meet physiological needs.

Total soil Fe ranged from 1505 mg/kg to 5935 mg/kg with a range of 99.8–1032 mg/kg in the leaves and 77.4—1565 mg/kg in the stems (Table 2). Site S5 (Shongweni), followed by S4 (Richards Bay), exhibited the highest soil Fe concentrations. The concentration of Fe in the plants from all sites was lower than the maximum permissible limit for Fe in plants (1000 mg/kg), which confirms the plant's ability to limit uptake, especially at elevated soil concentrations.[39] The Fe concentrations in this study were higher than those in the leaves from the plant obtained from a mountain in Yemen (177.3 mg/kg) and from fruits collected from Shada Mountain in Saudi Arabia (44.7 mg/kg).[17,18]

Higher levels of Cu were detected in the plant than in the soil, except at the environmentally polluted sites (S4 – Shongweni and S5 – Cato Ridge). Cu concentrations in the leaves and stems were mainly below the WHO permissible limit of 10 mg/kg for plants.[40] Cu concentrations obtained in this study (average of 5.1 mg/kg) were comparable to those reported in fruits (3.3 mg/kg), but relatively lower than those reported in leaves (11.6 mg/kg).[17,18] The low soil concentrations at most sites indicate Cu deficiencies, and the high BAFs provide evidence of the plants physiological control according to metabolic requirements, with plant root affinity for Cu rising when low soil Cu provides insufficient supply of the metal.

Generally, low concentrations of Co and Cr were detected in plant and soil samples from all sites with BAFs<1, indicating that the uptake of

these elements was restricted. If detected, Cr in plants ranged from 0.17 mg/kg (S1 – Margate) to 3.42 mg/kg (S6 – St Lucia) and 2.3–9.7 mg/kg in soil. Cr mainly exists in the innocuous trivalent form and noxious hexavalent form. An intake of 2– 5 g of Cr(VI) may be fatal. Total Cr in plant leaves at S6 (St Lucia) is within this range; therefore, consumers from this region should exercise caution as the hexavalent form is mobile and bioavailable under oxidising conditions and may contribute significantly to total concentration.[41] In this study, the average concentrations of the micronutrients in the plant were in decreasing order of Fe>Mn>Se>Zn>Cu>Cr>Ni>Co.

Arsenic concentrations in the soil ranged from 0.87 mg/kg (Cato Ridge) to 2.87 mg/kg (Umgababa), which exceeded the WHO maximum permissible limit of 0.5 mg/kg for As in agricultural soils.[42] The plant did not tend to accumulate As with BAFs<1 at most sites (Table 3). Arsenic in the plant leaves ranged from 0.44 mg/kg to 2.17 mg/kg, which is higher than the WHO/FAO maximum permissible limit of 0.1 mg/kg for plants grown on industrial soils.[43] A study reported the average As concentration in consumed vegetables to be 0.27 mg/kg for an industrial site and 0.05 mg/kg for a non-industrial site.[44] In our study, the average As in plant leaves was 1.18 mg/kg, which is higher than those previously reported. For Cd, the plant exhibited concentrations ranging from 0.57 mg/kg to 0.71 mg/kg in the leaves, which surpasses the maximum permissible limit of 0.2 mg/g for Cd in vegetables.[45] The concentration of Cd in the plant at all sites was comparable to that in soil (BAF almost 1), highlighting the plant's potential as an indicator for Cd pollution (Table 3).

Pb was generally higher in the soil than in the plant, with concentrations in the soil at S5 (Cato Ridge, 8.43 mg/kg) being extremely high relative to those at the other sites (Table 3). However, this did not influence the Pb concentration in the plant, which again indicates the plant's ability to protect itself by excluding toxic metals. The high soil concentrations at Cato Ridge could be due to vehicular emissions from a truck stop and railway line close to the sample collection point. The concentrations of Pb in the leaves from S1 (Margate) and S2 (Ballito) were 1.16 mg/kg and 1.06 mg/kg, respectively. According to the South African Department of Health, these values are above the maximum permissible limit of Pb of 0.3 mg/kg in leafy vegetables.[46] The data obtained for the toxic elements (As, Cd and Pb) suggest that *C. rotundifolia* samples collected from sites near roads and pollution sources are not suitable for human consumption. Although the plant limited uptake of these toxic metals, the concentrations obtained at some sites exceeded the maximum permissible limits imposed by authorities for safety for human consumption. The communities in these areas should exercise caution when consuming this plant to avoid toxic health effects.

Table 3: Concentrations of toxic elements (As, Cd and Pb) in *Cissus rotundifolia* leaves and surrounding soil from each site (mean (SD), n=3), estimated daily intake (EDI), target hazard quotient (THQ), carcinogenic risk (CR) and bioaccumulation factor (BAF)

Site	[Leaves] (mg/kg)	EDI	THQ	CR	[Soil] (mg/kg)	BAF [Leaves]/[Soil]
As						
S1	1.75 (0.39)	0.002	5.152	2.32E-03	1.88 (0.14)	0.9
S2	1.01 (0.66)	0.001	2.977	1.34E-03	2.67 (0.03)	0.4
S3	2.17 (0.07)	0.002	6.407	2.88E-03	1.605 (1.055)	1.4
S4	0.52 (0.82)	0.000	1.541	6.94E-04	1.87 (1.15)	0.3
S5	0.44 (0.125)	0.000	1.303	5.86E-04	0.87 (1.13)	0.5
S6	1.88 (0.925)	0.002	5.536	2.49E-03	2.835 (0.475)	0.7
S8	0.52 (0.635)	0.000	1.527	6.87E-04	2.865 (1.15)	0.2
Cd						
S1	0.64 (0.09)	0.001	0.562	3.54E-03	0.555 (0.065)	1.1
S2	0.63 (0.08)	0.001	0.554	3.49E-03	0.66 (0.04)	0.9
S3	0.68 (0.1)	0.001	0.602	3.79E-03	0.54 (0.03)	1.3
S4	0.68 (0.65)	0.001	0.598	3.77E-03	0.73 (0.05)	0.9
S5	0.64 (0.155)	0.001	0.567	3.57E-03	0.645 (0.035)	1.0
S6	0.63 (0.125)	0.001	0.558	3.52E-03	0.64 (0.63)	1.0
S7	0.65 (0.02)	0.001	0.571	3.60E-03	0.63 (0.085)	1.0
S8	0.75 (0.005)	0.001	0.660	4.16E-03	0.575 (0.06)	1.3
Pb						
S1	1.16 (0.015)	0.001	0.257	8.73E-06	0.975 (0.37)	1.2
S2	1.06 (0.99)	0.001	0.235	7.99E-06	0.34 (0.735)	3.1
S5	0.1 (0.002)	0.000	0.021	7.21E-07	8.415 (2.005)	0.0
S6	0.23 (0.085)	0.000	0.051	1.72E-06	0.96 (0.51)	0.2
S7	0.01 (0.145)	0.000	0.002	5.38E-08	0.61 (0.055)	0.0

S1, Margate; S2, Ballito; S3, Richards Bay; S4, Shongweni; S5, Cato Ridge; S6, St Lucia; S7, Amanzimtoti; S8, Umgababa

Table 4: The estimated contribution of essential elements in *Cissus rotundifolia* (leaves and stems) to dietary intake

DRI (mg/day)			Average concentration (mg/10 g dry mass)		Estimated contribution to RDA (10 g/day) %	
Element	RDA	UL	Stems	Leaves	Stems	Leaves
Ca	1300	2500	366.6	405.5	28.2	31.1
Cu	0.9	8	0.1	0.2	13.3	24.5
Fe	18	45	4.6	7.3	25.5	40.7
Mg	320	350	50.4	91.7	15.8	28.7
Mn	2.3	9	0.4	0.5	18.7	22.7
Se	0.055	0.4	0.4	0.7	669	1251
Zn	11	34	0.8	0.3	6.9	2.7

DRI, daily recommended intake; RDA, recommended dietary allowance; UL, tolerable upper intake level; ND, not detectable

The I_{geo} values obtained for the soil collected from the eight sites indicate that the soil was not contaminated with heavy metals. However, the health risk assessment showed possible non-carcinogenic or carcinogenic risks due to exposure to As and Cd. The THQs for the leaves were in the order of As>Cd>Pb and ranged from 1.30 to 6.41 for As, 0.55 to 0.66 for Cd, and 0.002 to 0.257 for Pb (Table 3). A THQ>1 for arsenic at all sites suggests possible non-carcinogenic risk to human health due to As exposure. The carcinogenic risk for As and Cd through consumption of the leaves was 1.57×10^{-3} and 3.68×10^{-3}, respectively. These values exceed the safe limit for cancer risk (1×10^{-4}) recommended by the USEPA[21], signifying the possibility of developing cancer over a lifetime if the plant is consumed frequently[23]. To the best of our knowledge, this is the first study to report on the metal toxicity of *C. rotundifolia*.

Contribution of C. rotundifolia to the human diet

The elemental concentrations of the leaves and stems were compared to the dietary reference intakes (DRIs) for most individuals to estimate the contribution of the consumption of 10 g of *C. rotundifolia* leaves and stems (based on the dry mass) to the diet (Table 4). For the elements to be considered good contributors to the diet, their concentrations should not exceed the tolerable upper intake levels (ULs).[33] Consumption of 10 g of leaves and stems would contribute 25.5% and 40.7% towards the RDA for Fe, respectively. Fe is an essential metal found in haemoglobin, and its deficiency in humans can lead to ailments such as anaemia, cancer, and heart disease.[47] Local people consuming this plant (especially those diagnosed with anaemia) can supplement their Fe intake, similar to consuming fruits and vegetables such as apricots, olives, and beetroot.[48]

The plant contributes >669% towards the RDA for Se, and the level in the leaves exceeded the UL. However, the concentrations of Se in the stems (0.37 mg/10 g) and leaves (0.69 mg/10 g) were similar to that of Brazil nuts (0.36 mg/10 g)[49], which are known to be a rich source of Se[49]. Se has antioxidant properties and has been reported to lower the risk of certain cancers, such as breast, lung, colon, and prostate cancers.[50] However, too much Se can have detrimental health effects and cause depression.[50] The other essential elements studied contribute adequately to their RDAs and may supplement the diet.

Conclusion

The analysis of *C. rotundifolia* revealed that the plant is rich in phytosterols and essential nutrients. The plant–soil system showed control by the plant to meet physiological needs. Bioaccumulation factors for Cd indicated the plant's potential as an indicator and biological monitor for Cd toxicity. Carcinogenic and non-carcinogenic risk assessments for As, Cd and Pb in the plant signified the possibility of developing cancer over time if consumed from exposed sites. From this study, it can be concluded that *C. rotundifolia* from non-polluted sites or sites away from roads, landfills, and other pollution sources is safe for human consumption. The findings from this study show the synergies when consuming medicinal plants and provide evidence for *C. rotundifolia* as a nutraceutical. Its consumption for therapeutic benefit would not pose metal toxicity risks, and for nutritional value would give the added advantage of ingesting antioxidants.

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Competing interests

We have no competing interests to declare.

Authors' contributions

B.P.M: Conceptualisation, methodology, data analysis, writing – the initial draft, writing – revisions, and student supervision. C.Z.B.: Methodology, writing – the initial draft, data collection, and data analysis. R.M.: Conceptualisation, methodology, data analysis, student supervision, project management, writing – revisions and funding acquisition.

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