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Antioxidant and Prebiotic Activity of Selected Edible Wild Plant Extracts

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Abstract

Edible wild plants were investigated as potential sources of antioxidants and prebiotics to benefit human health. Antioxidant activity, ascorbic acid and total dietary fibre contents were determined in edible wild plants from Lesotho, Swaziland and South Africa. Pure probiotic strains of *Bifidobacterium animalis* subsp. *animalis* (ATCC 25527), *Lactobacillus rhamnosus* (TUTBFD) and *Lactobacillus acidophilus* (ATCC 314) were cultured in broth containing edible wild plant extracts to assess their prebiotic activity. *Cyperus esculantus* had the highest arscobic acid content of 603±64.1 mg/100 g edible dry plant material followed by *Rosa rubiginosa* (500.8±48.8 mg/100 g). The two plants had IC_{50} of 10.7±0.2 µg/mL and 47.8±0.2 µg/mL for DPPH inhibition, respectively. Forty percent (40%) (n=30) of the edible wild plants had significant ($p<0.01$) total antioxidant activity ($IC_{50}<60$ µg/mL) and high ascorbic acid content (>200 mg/100 g). *Nasturtium officinale* reported the highest yield for soluble fibre (25%) while *Hypoxis hirsute* had the highest total dietary fibre content (7.3%). *Rorippa nudiuscula* enhanced the growth of *B. animalis* significantly (p=0.001), 8-fold more than inulin. *Chenopodium album* and *Urtica dioica* stimulated the growth of *L. rhamnosus* significantly (p=0.0001) than inulin, respectfully, while *Tragopogon porrifolius* significantly (p=0.0001) stimulated the growth of *L. acidophilus* than inulin. It was concluded that the investigated edible wild plants from southern Africa have antioxidant and prebiotic properties that may be beneficial to human health.

Keywords: antioxidant, Bifidobacterium, dietary fibre, edible wild plant, Lactobacillus, prebiotic, probiotic

1. Introduction

Edible wild plants provide energy, vitamins and minerals in the diet (Boedecker, Termite, Assogbadjo, Van Damme & Lachat, 2014). Reactive oxygen species, including superoxide anions, hydroxyl radicals, and hydrogen peroxide are generated in specific organelles of the cell under normal physiological conditions (Haraguchi, 2001). Excessive production of these reactive oxygen species and free radical mediated reactions are associated with degenerative diseases such as aging, cancer, coronary heart disease, and Alzheimer's disease (Sun, Wang, Fang, Gao & Tan, 2004). Consumption of sufficient amounts of fruits, vegetables and tubers leads to reduced incidences of heart disease, cancers and other degenerative diseases (Kaur & Kapoor, 2001). These foods provide an optimal mix of beneficial phytochemicals such as natural antioxidants, fibres and other biotic compounds (Kaur & Kapoor, 2001). According to Halliwell (1996), dietary compounds such as vitamins E and C are strong antioxidants while the antioxidant capacity of plant pigments (e.g. carotenoids), plant phenolics and flavonoids may involve other biological mechanisms such as increasing the expression of endogenous antioxidants. Some edible wild plant foods are also sources of vitamins and minerals. For example, a study by Bwembya, Thwala, Silaula and Otieno (2014) reported that Swazi wild edible vegetables like *Solanium nigrum* (Umsobo), *Corchorius olitorus* (Ligusha), *Momordica involucrate* (Inkhakha), *Amaranthus spinosus* (Imbuya) and *Bidens pilosa* (Chuchuza) are sources of nutrients such as pro-vitamin A, iron, calcium and zinc. Additionally, *Acacia senegal* (Gum Arabic) functionally increases the absorption of coenzyme Q10 (Ozaki et al., 2010).

Prebiotics are non-digestible food ingredients naturally present in some plant materials that stimulate the growth

and/or activity of 'friendly' probiotic bacteria in the digestive system in ways claimed to be beneficial to health (Gibson & Roberfroid, 1995). Prebiotic molecules can be divided into three categories, namely short-chain, long-chain and full spectrum prebiotics (Roberfroid, 2007). Inulin, oligofructose and lactulose have prebiotic effect and have been part of the human diet for centuries (Franck, 2002). The human intestine is densely populated with microorganisms that influence human biological processes as well as drug absorption (Kullberg, 2008). Some of these microorganisms originate from fermented foods or the environment and are known to have benefical effects on human health; hence the term probiotics (Holzapfel & Schillinger, 2002; Lei & Jakobsen, 2004). Microorganisms which are commonly used as probiotics belong to the heterogeneous group of lactic acid bacteria (Lactobacillus, Enterococcus) and to the genus Bifidobacterium (Food and Agriculture Organization of the United Nations and the World Health Organization [FAO/WHO], 2001; Ghosh, 2012; Holzapfel, Haberer, Geisen, Björkroth & Schillinger, 2001). The beneficial bacteria types include the well-known probiotic strains *B. animalis* subsp. *animalis*, *L. rhamnosus* and *L. acidophilus* (Jungersen et al., 2014; Weese & Anderso, 2002). Alvarez-Olmos and Oberhelman (2001) and Tien et al. (2006) described the mechanism of action for probiotics against gastrointestinal pathogens as based principally on: i) competition for nutrients and sites of adhession, ii) production of antimicrobial metabolites, iii) changing the gastrointestinal environmental conditions and, iv) modulation of the immune response of the host.

Probiotic bacteria have specific nutrient requirements and some of them are selectively stimulated by non-digestible carbohydrate molecules such as oligofructose (Gibson & Roberfroid, 1995; Quigley, 2010; Roberfroid, 2007). Previous studies reported that extracts from herbal products and dietary supplements act as prebiotics or bifidogenic factors by modulating the balance of human gut microbes (Roberfroid et al., 2010; Wang, Qi, Wang & Li, 2011). Probiotics have shown several benefits in human health (Parvez, Malik, Ah Kang & Kim, 2006). Some of the health benefits of probiotics include: i) cancer prevention (Baldwin et al., 2010), ii) reduction of *Helicobacter pylori* infection (Gotteland, Brunser & Cruchet, 2006; Pacifico et al., 2014), iii) reduction of cholesterol and triacylglycerol plasma concentrations (Jones, Martoni, Parent & Prakash, 2011), iv) beneficial effects on mineral metabolism (Lamberti et al., 2011), v) relief from constipation (Malaguarnera et al., 2012), vi) reduction of allergic symptoms (Matsuda et al., 2012), and vii) relief from irritable bowel syndrome (Thomson, Chopra, Clandinin & Freeman, 2012).

Antioxidants and prebiotics can be extracted from plants (e.g. ascorbic acid and inulin, respectively), by enzymatic hydrolysis (e.g. oligofructose from inulin), by synthesis (e.g. by trans-glycosylation reactions) from monosaccharides and disaccharides such as sucrose (fructooligosaccharides) and lactose (trans-galactosylated oligosaccharides or galactooligosaccharides) (Crittenden & Playne, 1996). Inulin and lactose oligosaccharides are the most studied prebiotics and have been recognized as dietary fibres in most countries (Moshfegh, Friday, Goldman & Chugahuja, 1999). Inulin is known to occur naturally in some plant species such as *Cichorium intybus* (Chicory), Jerusalem artichokes and to a lesser extent in onion, garlic, banana, asparagus, tomatoes and leek (Crittenden & Playne, 1996; Kassim, Baijnath & Odhav, 2014; Roberfroid, 2007). Among cereal grains, wheat is the best source of prebiotics, providing about 2.5 g fructooligosaccharides (FOS) and 2.5 g inulin (fructopolysaccharide) per 100 g raw bran (Van Loo, Coussement, De Leenheer, Hoebregs & Smits, 1995). Wheat is the major food source of naturally occurring inulin and FOS, providing about 70% of these compounds in American diets (Moshfegh et al., 1999).

In Lesotho, Swaziland, South Africa and southen Africa in general, fewer people in urban areas now use edible wild plants for nutrition due to the availability of domesticated food plants and fast foods (Hart & Vorster, 2006) while rural communities may depend more on these plants. Arguably, some domesticated food plants have low ascorbic acid content and antioxidant activity as compared to the edible wild plants (Legwaila, Mojeremane, Modisa, Mmolotsi & Rampart, 2011). Studies on edible wild plants from other geographical locations around the world largely focus on the proximate and micronutrient content analysis as well as agronomic improvement of wild and semi-wild crop varieties (Uprety et al., 2012). Meanwhile, southern African indigenous knowledge on the value of edible wild plants is rapidly eroding due to limited local scientific evidence to substantiate their benefit to human health. This paper reports on the ascorbic acid content, total antioxidant capacity and prebiotic activity of edible wild plants from Lesotho, Swaziland and the Limpopo province of South Africa to motivate for their benefits to human health, rational use and conservation.

2. Materials and Methods

2.1 Materials

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) dye and the ascorbic acid reference standard were purchased from Sigma-Aldrich (Johannesburg, South Africa). The 96 well UV/VIS microplates and HPLC grade methanol were

purchased from Anatech (Johannesburg, South Africa). Edible wild plants were sourced from the Lesotho, Swaziland and the Limpopo province of South Africa. De Man, Rogosa, Sharpe (MRS) broth, MRS agar, and petri dishes were purchased from Separations Scientific (Johannesburg, South Africa). Inulin, lactulose, α-amylase, protease, amyloglucosidase, *L. acidophilus* (ATCC 25527, Lot: 1092-05-1, France) and *B. animalis* subsp. *animalis* (ATCC 25527, Lot: 1092-05-1, France) were purchased from Sigma-Aldrich (Johannesburg, South Africa) while *L. rhamnosus* (TUTBFD) species was received from the Department of Biotechnology and Food Technology, Tshwane University of Technology. Diatomaceous earth flour (celite) and fritted-crucibles (porosity number 4 (14 μm)) all other materials were of analytical grade and were used as received.

2.2 Collection of Edible Wild Plants

Fruits, vegetables, roots, and tubers were among the samples collected. The fruits collected were *Opuntia megacantha*, *Rubus cuneifolius*, and *Rosa rubiginosa*. The vegetables were *Sonchus dregeanus*, *Rorippa nudiuscula*, *Amaranthus retroflexus*, *Chenopodium album*, *Urtica dioica*, *Wahlengergia androsacea*, *Lepidium capense*, *Tragopogon porrifolius*, *Sonchus oleraceus*, *Sonchus asper*, *Sisymbrium capense*, *Nemesia fruticans*, *Nasturtium officinale*, *Berkheya purpurea*, *Hypochaeris radicata*, *Sisymbrium thelungii*, *Sonchus integrifolius*, *Solanum retroflexum*, *Momordica foetida*, *Corchorus tridens*, *Amaranthus hybridus*, *Amarathus spinosus*, *Bidens pilosa*, and *Momordica involucrate*. *Cyperus esculentus* bulbs, *Hypoxis hirsute* and *Tragopogon porrifolius* taproot and *Discorea minutiflora* tubers were also collected.

2.3 Preparation of Plant Extracts

Local people in the areas from which the edible wild plants were collected usually chop the edible wild plants before consumption. Of interest was the simulation of these conditions and simple methods used for the determination of the amount of ascorbic acid left after the appropriate preparation method involving homogenizing, grinding with mortar and pestle, or shredding with a knife and natural drying. The fruits were homogenized in 200 mL of distilled water using a Kenwood kitchen food blender (Game, Pretoria, South Africa). The homogenate was frozen and lyophilized using a VirTis Benchtop K free-drier (Thermo Electron, Germiston, South Africa) at -50 ℃ for 48 hours. Plant leaves were air-dried at room temperature for 7 days and ground with a motor and pestle. Roots were shredded using a sharp knife then ground and ultra-sonicated for 24 hours at 25 ℃ in 500 mL distilled water, followed by filtration using Whatman filter paper pore size 0.45 μm (Sigma-Aldrich, Johannesburg, South Africa) into a volumetric flask. The remaining plant root material was ultra-sonicated with 300 mL distilled water at 30 ℃ with stirring for a further 12 hours. The two extract portions were combined to make 800 mL, which was frozen and lyophilized. The resultant powders were micronized (250 μm sieve mesh) and stored in labelled moisture-free airtight amber glass vials until further use (Tarirai, Viljoen & Hamman, 2012).

2.4 Determination of Ascorbic Acid Content

A 5 g amount of corn starch was added to 250 mL of distilled water in a beaker, which was heated with continuous stirring until the corn starch dissolved. The corn starch solution (5 mL) and distilled water (250 mL) were mixed in a 500 mL beaker to which four drops of a 0.05 M iodine solution were added and mixed thoroughly. The final iodine-corn starch dye solution was blue in color. The blue iodine-corn starch dye was standardized by volume using the mass of the ascorbic acid standard. The standard was prepared by dissolving ascorbic acid (5 mg) in 10 mL of distilled water. The ascorbic acid solution was poured into a burette and titrated into a 5 mL volume of the blue iodine-corn starch dye to a colorless endpoint that persisted for at least 15 seconds. The amount of ascorbic acid required to turn the 5.0 mL volume of the blue iodine-corn starch dye to colorless was calculated by using equation (1):

$$M_1 = VM/V_1 \quad (1)$$

Where M_1 (mg) is the amount of ascorbic acid contained in a titrated volume, V_1 (mL), used to reduce 5 mL of the dye from blue to colorless. M (mg) is the known amount of ascorbic acid that was weighed off and dissolved into the total volume (V, mL) of ascorbic acid stock solution i.e. M plus mass of distilled water.

Edible wild plant extracts were prepared by dissolving a 1 g of crude plant extract powder in 20 mL of distilled water (i.e. 50 mg/mL before filtration). The mixture was left for 30 minutes at room temperature and then filtered using Whatman filter paper pore size 0.2 μm (Sigma-Aldrich, Johannesburg, South Africa). For the qualitative determination of the presence of ascorbic acid in a plant sample, the aqueous plant extracts were slowly titrated into the standardised 5 mL volume of iodine-corn starch solution until the blue solution became clear or the plant extract solution (10 mL) got depleted. For the quantitative assay of ascorbic acid, the volume of the plant extract required to turn the blue iodine-corn starch dye to colorless was recorded. The concentration of ascorbic acid in

the plant extract aliquot titrated against 5 mL standardized dye was proportionally determined using equation (2):

$$M_2=V_1M_1/V_2 \quad (2)$$

Where M_2 is the amount of ascorbic acid (mg) in the aliquot of crude plant extract contained in a V_2 (mL) of the plant extract aliquot titrated into 5 mL of the dye. M_1 is the mass of ascorbic acid in the volume V_1 of ascorbic acid solution used to reduce 5 mL of dye from blue to colorless from equation (1).

The amount of ascorbic acid in the total volume of crude plant extract prepared was obtained using equation (3):

$$M_t=V_2M_2/V_t \quad (3)$$

Where M_t (mg) is the total amount of ascorbic acid in the total stock volume (V_t, mL) of crude plant extract prepared. M_2 (mg) is the amount of ascorbic acid in an aliquot (V_2, mL) of crude plant extract titrated, to endpoint, into 5 mL of dye from equation (2).

The ascorbic acid content of each plant extract was determined using equation (4) and was expressed as milligrams of ascorbic acid per 100 grams of plant material:

$$Ascorbic\ acid\ content = (M_t \times 100)/M_s \quad (4)$$

Where M_t (g) is the total amount of ascorbic acid in a known amount (M_s, g) of crude plant extract used to prepare the plant extract stock solution.

2.5 Evaluation of Total Antioxidant Capacity

About 100 g of each freeze-dried, ground and micronized plant material was soaked in 1 L of high performance liquid chromatography (HPLC) grade methanol 98% v/v for 5 days and was stirred every 18 hours using a sterilized glass rod at room temperature. The final extracts were passed through Whatman filter paper pore size 0.2 µm (Sigma-Aldrich, Johannesburg, South Africa). The filtrates obtained were concentrated under vacuum using a Rotavapor R-200 (Buchi Labortechnik AG, Flawil, Switzerland) at 40 ^oC until the methanol evaporated. The samples were wetted with 10 mL of distilled water, lyophilized and stored at 4 ^oC for further use (Khalaf, Shakya, Al-Othman, El-Agbar & Farah, 2008).

Stock solutions were prepared by dissolving 5 mg of dry filtered plant extract in 1 mL of 98% v/v HPLC grade methanol. Working solutions of the extracts were appropriately diluted from the stock solutions to obtain concentrations of 10, 50, 100, 250, and 500 µg/mL). Ascorbic acid was used as a reference across a 10-500 µg/mL concentration range. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) dye was prepared in HPLC grade methanol to obtain a 0.002% w/v DPPH dye solution.

A 150 µL aliquot of each concentration of filtered plant extract solution and 150 µL of ascorbic acid solution were separately plated (in triplicates) in a 96-well microplate followed by 150 µL of DPPH solution added to each well. The microplate was placed in a SpectrostarNano UV/Vis microplate reader (BMG LabTech, Ortenberg, Germany) and shaken for 10 seconds to mix its contents. The assays were kept in the dark for 30 minutes and their optical densities or UV/Visible absorbance was measured at 517 nm. Methanol (150 µL) with DPPH solution (0.002%, 150 µL) was used as a blank. The optical density was recorded and the % inhibition of DPPH activity was calculated using equation (5):

$$\%\ inhibition\ of\ DPPH\ activity = (A_{test\ sample}/A_{blank}) \times 100 \quad (5)$$

Where A = optical density of the test sample or the blank (Khalaf, Shakya, Al-Othman, El-Agbar & Farah, 2008). The radical scavenging effect of test samples was measured as a decrease in the absorbance of DPPH over time. The purple color of the reaction mixture changed to yellow and the UV/Visible absorbance at 517 nm decreased in the presence of increasing antioxidant activity (Tung, Wu, Kuo & Chang, 2007). Plots (not shown) of the % DPPH inhibition (y-axis) and the concentration of plant extract (x-axis) were used to extrapolate the concentration (IC_{50}, ug/mL) at which 50% inhibition of DPPH occurred for each plant extract.

2.6 Extraction of Prebiotics

The prebiotic carbohydrates (soluble fibre) were extracted according to an adaptation of the method described by Iwata, Hotta and Goto (2009). The powders were extracted in duplicate for each sample. The dried powder samples were soaked in 50% v/v ethanol and left at room temperature for 3 days. The samples were then filtered through a Whatman No. 1 filter paper. The filtrates were freeze-dried (VirTis Benchtop K, Model 2KBTES, SP industries) at -50 ℃ for 18 hours) after concentrating using a rotary evaporator (Model R-200 Buchi Rotavapor, Netherlands) at 200 rpm at 50 ℃ for 20 minutes). The freeze-dried extracts of edible wild plants were preserved in airtight glass containers until further used. The % yield of the plant extracts was determined using equation (6) as described by Wichienchot et al. (2011):

$$Yield\ (\%) = (weight\ dried\ extract\ (g)\ x\ 100)\ /\ initial\ amount\ (g) \quad (6)$$

2.7 Determination of Total Dietary Fibre Content

Digestion in acid and enzyme media was used to separate the digestible fibre from the indigestible material. The acid digestion test was done according to the method(s) described by Iwata et al. (2009) and Wichienchot et al. (2011). Solutions (10% w/v) of the dried extracted carbohydrates were made in distilled water by dissolving a 250 mg ethanol-based sample of the crude plant extract from Section 2.3 in 2.25 mL distilled water. The mixture was incubated with a 225 µL HCl buffer of pH 1 at 37 ℃ for 4 hours. The reaction was terminated by neutralization using a 225 µL of 1 N sodium hydroxide solution. The acid-digested samples were diluted with a 12.5 mL phosphate buffer (20 mM) at pH 6.0 and the final volume was 15.2 mL. The pH of this final sample was adjusted to 6.0.

For enzymatic digestion, the acid-digested solutions were treated according to a modified AOAC method 985.29 (AOAC International, 1995). The acid-digested samples were incubated with 30 µL of 3000 units/mL α-amylase at 50 ℃ for 60 minutes in a water bath. The samples were cooled to room temperature and the pH was adjusted to 7.5. This was followed by digestion with 30 µL of 50 mg/mL protease at 60 ℃ for 30 minutes in the water bath. The samples were cooled to room temperature and their pH was adjusted to 4.0. The samples were further incubated with 75 µL of amyloglucosidase at 60 ℃ for 30 minutes in the water bath. The pH of the samples was measured using a SensION^{TM} $^{+}$PH31 meter (HACH, Serial no.: 415006, Spain) throughout the experiment. When the enzymatic digestion sequence was completed, 60 mL of 95% v/v ethanol previously heated to 60 ℃, was added to each sample. The solutions were left to precipitate over 12 hours at room temperature and then filtered using fritted-crucibles, porosity number 4 (14 µm) covered with diatomaceous flour (celite) (particle size 16 µm) bed under vacuum. The fritted-crucibles were first cleaned and 0.5 g diatomaceous flour was weighed off in each crucible. The flour was wetted with 78% v/v ethanol followed by vacuum application to get rid of the ethanol thus forming a thin bed of diatomaceous flour over the base of each crucible. The fritted-crucibles containing the diatomaceous flour bed were then heated at 130 ℃ in an oven. After heating, the crucibles were cooled in a desiccator, weighed off and used to filter the precipitated enzymatic digested samples.

The residues inside the fritted-crucibles were dried over 12 hours. After the drying process, each fritted-crucible with its contents was weighed and its mass was recorded. One duplicate set of the residues was analysed for protein content using a TruMac® N Nitrogen determinator Version 1.20 (Part number: 200-739, LeCo, USA). The second duplicate set of residue was used to determine ash content by incinerating in the oven at 320 ℃ for 8 hours. The residue weight, an average of duplicate samples, was calculated by subtracting the mass of the crucible plus diatomaceous flour from the mass of crucible plus diatomaceous flour plus sample precipitate. This residue weight, minus the protein and ash residue weights, represented the weight of insoluble dietary fibre and high molecular weight soluble dietary fibre. Protein content of the residue was calculated using equation (7):

$$Protein\ content = N\ x\ 6.25 \quad (7)$$

Where *N* is the nitrogen content of the residue and 6.25 is the protein conversion factor.

Total dietary fibre content was calculated using equation (8):

$$Total\ dietary\ fibre = weight\ (residues) - (protein + ash) \quad (8)$$

The percentage total dietary fibre was calculated using equation (9):

$$\%\ Total\ dietary\ fibre = (Total\ diatary\ fibre\ x\ 100)/weight\ of\ test\ sample \quad (9)$$

Where weight residue = average of duplicates, and weight test portion = average of duplicates.

2.8 Assessment of Prebiotic Activity

2.8.1 Cultivation of Bacteria Cultures

Pure strains of *B. animalis* subsp. *animalis* (ATCC 25527, Lot: 1092-05-1, France) and *L. acidophilus* (ATCC 314, Lot: 885-34-11, France) were activated by aseptically inoculating them into sterile MRS broth (5 mL). Aliquots (1.5 mL) of each of the activated cultures were separately transferred to fresh MRS broth (15 mL) in centrifuge tubes. Each treatment was done in triplicate. *B. animalis* and *L. rhamnosus* containing tubes were incubated anaerobically (Anaerocult gas mixture, Merk, SA) for 48 hours at 37 ℃, while the *L. acidophilus* was incubated in a 5% carbon dioxide atmosphere at 37 ℃ for 48 hours. After incubation, the tubes were centrifuged at 277 *x*g in a 5417C/R centrifuge (Epperndorf, Germany) for 15 minutes to obtain a pellet of bacteria. The broth was aseptically decanted and resuspended in MRS broth (5 mL) containing 20% glycerol. The pellets were then stored at below -20 ℃ until required for further tests.

2.8.2 Reactivating Frozen Bacterial Cultures

A loopful of thawed bacterial culture was streaked onto MRS agar (Biolab, South Africa). *B. animalis* and *L. rhamnosus* were incubated anaerobically at 37 ℃ for 48 hours, while *L. acidophilus* was incubated in a 5% carbon dioxide atmosphere at 37 ℃ as previously described in section 2.8.1.

2.8.3 Preparation of Test Solutions and Inoculation of Bacterial Cultures

A solution (2.5 mL) containing 5 mg/mL of plant extract for each of the selected edible wild plants was transferred to MRS broth (10 mL) in triplicates. The tubes were thoroughly mixed using a vortex mixer and sterilized at 121 ℃ for 15 minutes. After cooling, the tubes were inoculated with a loopful of *B. animalis*, *L. rhamnosus*, or *L. acidophilus*. *B. animalis* and *L. rhamnosus* were incubated anaerobically 37 ℃ for 24 hours as previously described in section 2.8.2, while *L. acidophilus* was incubated under 5% carbon dioxide.

2.8.4 Enumeration of Bacteria

The bacterial macroculture counts for inoculated test broth were enumerated on MRS agar using the drop plate technique (Herigstad, Hamilton & Heersink, 2001; Munsch-Alatossava, Rita & Alatossava, 2007; Naghili et al., 2013). *B. animalis* and *L. rhamnosus* were incubated anaerobically at 37 ℃ for 72 hours, while *L. acidophilus* was incubated in 5% carbon dioxide/oxygen.

2.9 Statistical Analysis of Data

Ascorbic acid content (mg/100 g of dry edible plant material), total antioxidant activity (ug/mL) and the log (bacterial count/mL) were determined in the presence of plant extracts. One-way analysis of variance (ANOVA) was peformed using MS Excel-*XLSAT* (Microsoft, Redmond, USA) to determine the differences in ascorbic acid content, antioxidant and prebiotic activity between plant extracts and also in comparison with the controls. Significant differences were taken for values of $p<0.01$.

3. Results and Discussion

3.1 Percentage Yield of Soluble Dietary Fibre

The results for the percentage yields of ethanolic extracts of edible wild plants are presented in Table 1. *Rosa rubiginosa* had the highest percentage yield of 49.4% while *Discorea minutiflora* species had the lowest percentage yield of 7.8%.

3.2 Antioxidant Activity of Edible Wild Plants

Cyperus esculentus showed the highest potency (IC_{50}=10.7±0.2 μg/mL) and corresponding high percentage inhibition of DPPH (data not shown) followed by *Berkheya purpurea* (IC_{50}=11.9±0.1 μg/mL) while *Discorea minutiflora* (IC_{50}=338.6±0.3 μg/mL) had the lowest capacity (Table 1). The antioxidant activity of the edible wild plants showed a trend similar to that observed for the values of ascorbic acid content (Table 1) for 87% (n=30) of the edible wild plants. This suggests that the antioxidant activity observed in the plant extracts was partly due to ascorbic acid.

Other antioxidants previously reported in edible wild plants and herbs include tocopherols and carotenoids (Choi, Jeong & Lee, 2007); tannins (Lee, Koo & Min, 2004); phenols and flavonoids (Nair, Nagar & Gupta, 1998). These phytochemicals partly contributed to the total antioxidant activity reported as indicated by the significantly ($p<0.01$) low IC_{50} values obtained for those plant extracts which had low ascorbic acid content notably *Rorippa nudiuscula* (IC_{50}=22.6±0.2 ug/mL, but had 77.0±3.0 mg/100 g ascorbic acid) and *Sonchus integrifolus* (IC_{50}=19.1±0.3 ug/mL with 80.2±8.4 mg/100 g ascorbic acid). Conversely, *Amaranthus hybridus* (IC_{50}=233.0±1.0 ug/mL) and *Momordica involucrate* (IC_{50}=146.6±0.3 ug/mL), which scored fairly high in ascorbic acid content showed weak antioxidant activities possibly due to low content of tocopherols, carotenoids, tannins, phenolics and flavonoids. The content of these phytochemicals in the edible wild plant extracts need further investigation. Additionally, all the three *Sonchus* subspecies included in the currect study consistently showed high ascorbic acid content and potent antioxidant activity.

Ozen (2010) reported that DPPH inhibition by aqueous wild edible plant extracts (100 μg/mL) from Turkey ranged from 48% to 78%. *Amaranthus retroflexus* aqueous extract caused a 69% DPPH inhibition while the methanolic extract (100 μg/mL) of the same plant elicited 43% in the present study. *Momordica foetida* leaves showed 45% inhibition of the superoxide anion at 175 μg/mL in an Italian study conducted by Acquaviva et al. (2013) while inhibition of DPPH activity by the same plant was 55% at 175 μg/mL in this study. Gacche, Kabaliye, Dhole and Jadhav (2010) reported that the DPPH scavenging activity of 1 mg/mL common vegetable extracts ranged from 20% to 67% while that of edible wild plant extracts at 500 μg/mL ranged from 53% to 100% in the present study.

Table 1. Percentage Yield of Soluble Fiber, Total Antioxidant Capacity and Ascorbic Acid Content of the Edible Wild Plant Extracts

Description of plant sample				**% yield of soluble fibre**	**Total antioxidant activity**[+]	**Ascorbic acid content**[+]
Plant botanical name	Plant common English name	Plant vernacular name[#]	Part of plant used	(mean ±sd, n=2)	IC_{50}, µg/mL (mean ±sd, n=3)	mg/100 g (mean ±sd, n=3)
Ascorbic acid	Vitamin C				3.5 ±0.2[a]	
Rubus Cuneifolius	Wild berries/ Sand bramble	Monokots'oai (Ss)	Ripe fruits	39.5 ±3.2	30.2 ±0.3[c]	298.5 ±24.3[e]
Opuntia megacantha	Prickly pears	Torofeiea	Ripe fruits	47.0 ±4.8	348.9 ± 0.4	NT[*]
Sonchus dregeanus	Thistle	Leharasoana (Ss)	Young leaves	16.4 ±1.6	18.1 ±0.2[b]	128.5 ±6.5
Rorippa nudiuscula	-	Papasane (Ss)	Young leaves	20.0 ±2.1	22.6 ±0.2[b]	80.2 ±8.4
Rosa rubiginosa	Rosehip	'Morobei (Ss)	Ripe fruits	49.4 ±4.9	47.8 ±0.2[e]	500.8 ±48.8[f]
Amaranthus retroflexus	Red pigweed	Thepe (Ss)	Young leaves	19.3 ±0.7	139.3 ±0.6	132.8 ±12.1
Chenopodium album	Wild spinach	Seruoe (Ss)	Young leaves	19.6 ±0.8	77.8 ±0.2	240.5 ±16.5[b]
Urtica dioica	Sting nettle plant	Bobatsi (Ss)	Young leaves	13.3 ±0.2	89.0 ±0.1	205.5 ±13.8[a]
Wahlengergia androsacea	Hare-bell	Tenane (Ss)	Young leaves	12.2 ±0.1	121.8 ±0.4	273.0 ±19.9[d]
Lepidium capense	Cape pepper cress/weed	Qhela (Ss)	Young leaves	12.7 ±0.3	122.2 ±1.0	160.8 ±16.1
Tragopogon porrifolius	Salsify	Moetse-oa-pere (Ss)	Young leaves, taproot	13.1 ±0.4	33.4 ±0.6[c]	240.5 ±16.5[b]
Sonchus oleraceus	Sowthistle	Leshoabe (Ss)	Young leaves	20.1 ±4.6	35.2 ±0.4[d]	121.4 ±10.6
Sonchus asper	Sowthistle	Leshoabe (Ss)	Young leaves	15.0 ±2.1	54.2 ±0.4[f]	287.0 ±38.3[d]
Sisymbrium capense	-	Tlhako ea khomo (Ss)	Young leaves	13.9 ±0.8	181.5 ±0.5	NT[*]
Nemesia fruticans	Wild nemesia	Malana a konyana (Ss)	Young leaves	20.2 ±3.5	48.5 ±0.5[e]	261.5 ±19.9[c]
Nasturtium officinale	Watercress	Semetsing/Selae (Ss)	Young leaves	25.0 ±4.7	82.6 ±0.4	123.0 ±8.4
Berkheya purpurea	Purples	Sehlohlo (Ss) (Ss)	Young leaves	20.3 ±2.1	11.9 ±0.1[a]	298.5 ±24.3[e]
Hypochaeris radicata	Cat's ear	Lepheo-la-khoho (Ss)	Young leaves	15.3 ±1.8	42.2 ±0.3[d]	155.0 ±8.6

Note. [#]Ss=Sesotho, V=Venda, Sw=Swati. [*]NT = Not tested (no ascorbic acid based on preliminary qualitative test, data not shown). [+]Different letters indicate significant statistical difference ($p<0.01$) for total antioxidant activity and ascorbic acid content values of edible wild plant extracts.

Table 2. Percentage Yield of Soluble Fiber, Total Antioxidant Capacity and Ascorbic Acid Content of the Edible Wild Plant Extracts (continued)

Description of plant sample				**% yield of soluble fibre**	**Total antioxidant activity**[+]	**Ascorbic acid content**[+]
Plant botanical name	Plant common English name	Plant vernacular name[#]	Part of plant used	(mean ±sd, n=2)	IC_{50}, µg/mL (mean ±sd, n=3)	mg/100 g (mean ±sd, n=3)
Sisymbrium thelungii	Wild mustard	Sepaile (Ss)	Young leaves	22.2 ±2.6	101.0 ±0.5	121.4 ±10.6
Cyperus esculentus	Sedge/ Earth Almond	Monakalali (Ss)	Bulb	22.8 ±1.9	10.7 ±0.2[a]	603.0 ±64.1[f]
Hypoxis hirsute	-	Lentsikitlane (Ss)	Taproot	9.6 ±0.1	334.5 ±0.5	NT[*]
Sonchus integrifolius	Thistle	Sentlhokojane (Ss)	Young leaves	20.8 ±0.9	19.1 ±0.3[b]	77.0 ±3.0
Solanum retroflexum	Wonderberry/nightshade	Muxe	Young leaves	14.8 ±0.4	198.9 ±0.2	172.1 ±5.6
Momordica foetida	-	Nngu (V)	Young leaves	15.5 ±0.4	155.1 ±0.3	116.0 ±4.9
Corchorus tridens	Wild jute plant	Delele (V)	Young leaves	10.4 ±0.3	157.5 ±0.5	68.5 ±7.0
Amaranthus hybridus	Pigweed	Thebe (V)	Young leaves	18.7 ±0.8	233.0 ±1.0	212.4 ±8.4[a]
Amarathus spinosus	Pigweed	Imbuya (Sw)	Young leaves	9.8 ±0.3	121.1 ±0.6	109.8 ±4.1
Bidens pilosa	Black jack	Chuchuza (Sw)	Young leaves	11.5 ±0.3	193.2 ±0.4	66.1 ±0.9
Momordica involucrate	Bitter gourd	Inkhakha (Sw)	Young leaves	23.8 ±1.6	146.6 ±0.3	224.1 ±6.5[a]
Discorea minutiflora	Yam	Emadhumbe (Sw)	Tubers	7.8 ±0.2	338.6 ±0.3	20.0 ±1.0

Note. [#]Ss=Sesotho, V=Venda, Sw=Swati. [*]NT = Not tested (no ascorbic acid based on preliminary qualitative test, data not shown). [+]Different letters indicate significant statistical difference ($p<0.01$) for total antioxidant activity and ascorbic acid content values of edible wild plant extracts.

3.3 Ascorbic Acid Content of the Edible Wild Plants

A total of 27 (90%) edible wild plants contained ascorbic acid ranging from 20.0 ±1.0 to 603 ±64.1 mg per 100 g edible portion of dry plant material (Table 2). *Cyperus esculantus* had the highest ascorbic acid content of 603 ±64.1 mg/100 g followed by *Rosa rubiginosa* at 500.8 ±48.8 mg per 100 g.

Local people in the areas from which the plant samples were collected usually chop the edible wild plants before consumption. These conditions were simulated to determine the amount of ascorbic acid left after such simple, but harsh preparation procedures like homogenizing, grinding with mortar and pestle, or shredding with a knife. Despite these harsh treatments, the ascorbic acid content of *Rosa rubiginosa* in this study correlates well with previous reports. For example, Ropciuc, Cenusa, Caprita and Cretescu (2011) reported ascorbic acid content ranging between 347.1 and 621.3 mg/100 g for *Rosa rubiginosa*, which varied with altitude, type of soil and humidity. Ascorbic acid content of *Rosa rubiginosa* was at 500.8 ±48.8 mg per 100 g in the current study.

Conversely, Pradhan, Manivannan, and Tamang (2015) reported the ascorbic acid content of 44.00 ±0.17 mg/100 g for *Amaranthus viridis*, 7.0 ±0.1 mg/100 g for *Urtica dioica*, and 3.0 ±0.1 mg/100 g for *Chenopodium album* while the ascorbic acid of *Amaranthus* species in the present study ranges from 109.8 to 212.4 mg/mL, 205.5 mg/mL for *Urtica dioica*, and 240.5 mg/mL for *Chenopodium album*. Difference in results from the two studies

is explained in terms of the different geographical locations (i.e. India and Southern Africa), which inherently give rise to different effects from altitude, climate, and soil type/fertility on plant composition as well as the methods of plant preparation and handling.

A frequency distribution of the edible wild plants investigated in this study, categorized by ascorbic acid content, antioxidant activity and the perceived potential societal value, is presented in Table 3. Approximately 87% (n=30) of the edible wild plants had adequate (>60 mg/100 g) ascorbic acid content according to the Food and Drug Administration [FDA] (2016). *Cyperus esculentus* and *Rosa rubiginosa* and ten other edible wild plants had significantly ($p<0.01$) high ascorbic acid content (>200 mg/100 g edible plant material) and potent total antioxidant activity (IC_{50}<60 ug/mL). *Cyperus esculentus*, *Rosa rubiginosa* and the *Sonchus* species are potential commercial sources of ascorbic acid and antioxidants.

Many rural communities in southern Africa depend on edible wild plants as famine foods. People even preserve these edible wild plants by chopping, then air and/or sun drying them for later use during the dry seasons (i.e. April to August). Plants tend to lose their ascorbic acid when their tissues are ground without being immersed in 5% metaphosphoric acid. However, the present study reports adequate amounts of ascorbic acid of >60 mg/100 g edible portion of plant material as stipulated by the FDA (FDA, 2016) were retained in 87% (n=30, Table 3) of the edible wild plants prepared by indigenous southern African methods.

Table 3. Classification of Edible Wild Plants Based on their Potential Antioxidant Value

Potential value of edible wild plants	**Ascorbic acid content***	**Total antioxidant activity****	**% (n=30)**
	mg/100 g of plant extract	IC_{50} (μg/mL) of plant extract	
No/low value category Edible wild plants with no or low value with respect to ascorbic acid content and total antioxidant activity.	0	>300	10
***Limited value category* Generally meets the FDA daily value (DV) for ascorbic acid (60 mg), but has limited antioxidant value.	≤60	201-300	3
Average value category Average ascorbic acid and moderate total antioxidant activity. Edible wild plants with potential for use both as daily sources of ascorbic acid and antioxidants as well as for therapeutic purposes.	61-200		47
+*High value category* Edible wild plants with high ascorbic acid content and high total antioxidant capacity. Edible wild plants that potentially have high therapeutic and high commercial value e.g. *Rosa rubiginosa* fruit and *Cyperus esculentus* bulb.	≥201	≤60	40

Note. *A daily value (DV) of 60 mg for dietary ascorbic acid consumption (FDA, 2016) was used as reference for *high value*. **IC_{50} = Concentration (ug/mL) of plant extract (ascorbic acid and phenolic) that induced 50% inhibition of 0.002% w/v DPPH activity.

Notably, vegetables are usually cooked before consumption, unless used in a salad, and this can affect the ascorbic acid content in the final dish. Paul and Ghosh (2012) reported that degradation of pure ascorbic acid and pomegranate fruit (*Punica granatum*) increased with an increase in temperature (during cooking), but the degradation of pure ascorbic acid was higher than that of the pomegranate fruit. The plant cell materials, including insoluble starch and celluloses fibers, in pomegranate and edible wild plants prepared would shield the ascorbic acid from overheating thus slowing the degradation process. Future studies can explore the ascorbic acid content of final dishes for those edible wild plants from this study, which are prepared through cooking.

In comparison, Venkatachalam, Rangasamy and Krishnan (2014) determined the ascorbic acid content of cultivated common vegetables and fruits from southern India. The ascorbic acid content of cultivated common vegetables such as mulberries, papaya, red grapes, guava, tomato, red onion, red cauliflower, carrot, and beetroot ranged from 10.83 to 68.71 mg/100 g. The values are low when compared to those of edible wild plants investigated in the present study. These findings support the viewpoint that edible wild plants are arguably better sources of ascorbic acid than cultivated plants (Legwaila et al., 2011). Most cultivated plants are genetically modified cultivars, and theoretically, they tend to grow faster before taking up enough nutrients from the soil. On the other hand, edible wild plants slowly grow in natural habitats thus gaining sufficient nutrients by their late maturity state.

3.4 Total Dietary Fibre Content of Edible Wild Plants

Table 4 presents the results for total dietary fibre content in the edible wild plant extracts. The total dietary fibre in the edible wild plants ranged from 7.97 mg/g to 73.48 mg/g with *Hypoxis hirsute* having the highest amount of total dietary fibre (7.3%) followed by *Amarathus spinosus* with 5.5%, and *Momordica involucrate* with 4.8%. The lowest amount of total dietary fibre (0.8% or less) was observed for *Sisymbrium capense*. *Rorippa nudiuscula*, *Sonchus dregeanus*, *Rosa rubiginosa*, *Urtica dioica*, *Berkheya purpurea*, *Sisymbrium thelungii*, *Cyperus esculentus*, *Corchorus tridens*, *Amaranthus hybridus* and *Bidens pilosa* also had higher amounts of total dietary fibre.

An ideal prebiotic must escape acidic and mammalian enzyme digestion in the upper gastrointestinal tract so that it can be released in the lower gastrointestinal tract and be used by the beneficial microorganisms in the colon, mainly bifidobacteria and lactobacilli (Gibson, Probert, Loo, Rastall & Roberfoid, 2004). Prebiotics are non-digestible carbohydrates like dietary fibre which are selectively fermented by probiotics (Iwata et al., 2009). The total dietary fibre content of edible wild plants indicates the undigested portion of plant material that can reach the lower gastrointestional tract, where bifidobacteria and lactobacilli are densely populated. Edible wild plants with high total dietary fibre content are expected to stimulate the growth of probiotics better than edible wild plants with low amounts of total dietary fibre. However, there are several other factors than total dietary fibre alone, such as reducing sugars, which can be contributing to the stimulatory effects of edible wild plants.

3.5 Prebiotic Activity of Edible Wild Plants

3.5.1 Effects of Edible Wild Plant Extracts on the Proliferation of *B. Animalis* Subsp. *Animalis* (ATCC 25527)

The colonies of *B. animalis* were observed as white, glistering and medium in size. The bacteria were short, rod-shaped and gram positive. The stimulation of *B. animalis* growth by edible wild plants ranged from log (cfu/mL)=7.00±0.01 for *Discorea minutiflora* to log (cfu/mL)=8.15±0.05 in the presence of *Rorippa nudiuscula* after 48 hours as presented in Table 4. Twenty-two edible wild plants stimulated the growth of *B. animalis* much better as compared to the broth (negative control, log (cfu/mL)=7.52±0.07 and inulin (positive control, log (cfu/mL)=7.75±0.05. Other plants that markedly stimulated the growth of *B. animalis* better than the positive control were *Rubus cuneifolius*, *Sonchus dregeanus*, *Amaranthus retroflexus*, *Chenopodium album*, *Urtica dioica*, *Wahlengergia androsacea*, *Lepidium capense*, *Nemesia fruticans*, *Berkheya purpurea*, *Taraxacum officinale*, *Sisymbrium thelungii*, *Cyperus esculentus*, *Sonchas integrifolius*, *Solanum retroflexum*, *Corchorus tridens*, *Amaranthus hybridus* and *Amarathus spinosus*.

Most of these plants stimulated the net growth of *B. animalis* several times better than inulin (positive control). For example, *Rorippa nudiuscula* enhanced the growth of *B. animalis* 11 times more than inulin, *Sonchus dregeanus* by 9-fold more, with *Sisymbrium thelungii* at 7-fold, and *Urtica dioica* was 6-fold more effective than inulin. The observed high growth stimulatory effects of these edible wild plants could be due to the presence of high total dietary fibre, reducing sugars or inulin. *Rorippa nudiuscula* had high total dietary fibre content, which correlates with its higher stimulatory effects on the growth of *B. animalis* and so were *Sisymbrium thelungii* and *Urtica dioica*. Kassim et al. (2014) reported that *Solanum nigrum* (leaves), *Momordica balsamina* (leaves), *Amaranthus spinosus* (leaves), *Amaranthus hybridus* (leaves), *Amaranthus dubius* (leaves), *Sonchus oleraceus* (leaves and roots), *Taraxacum officinale* (leaves and roots) had a higher growth stimuli on probiotics. The same edible wild plants from different geographical locations also showed high stimulation on growth of *B. animalis* in the present study. Sreenivas and Lele (2013) reported that gourd family vegetables had significant prebiotic ability on *B. breve*. Similarly, in the present study, *Momordica foetida* stimulated the growth of *B. animalis*. However, *Momordica involucrate*, which had appreciable total dietary fibre content, inhibited the growth of all three probiotics indicating that the edible wild plants probably possesses some antimicrobial activity.

3.5.2 Effects of Edible Wild Plant Extracts on the Proliferation of *L. Rhamnosus* (TUTBFD)

The colonies of *Lactobacillus rhamnosus* were white, glistering and of medium size. The bacteria were gram positive rods. Log colony counts of *L. rhamnosus* ranged from log (cfu/mL)=8.29±0.03 for *Amaranthus hybridus* to log (cfu/mL)=8.85±0.01 in the presence of *Urtica dioica* as shown in Table 4. Twenty-three edible wild plants stimulated the growth of *L. rhamnosus* better than both the broth (negative control, log (cfu/mL)=8.51±0.01 and the positive control i.e. inulin, log (cfu/mL)=8.58±0.01. Other plants that demonstrated stimulatory effects on the growth of *L. rhamnosus* include *Rorippa nudiuscula*, *Rosa rubiginosa*, *Chenopodium album*, *Lepidium capense*,

Table 4. Effects of Soluble Fibres from Edible Wild Plants on the Growth of *Bifidobacterium animalis* subsp. *animalis (*ATCC 25527), *Lactobacillus rhamnosus* (TUTBFD) and *Lactobacillus acidophilus* (ATCC 314)

Test sample	**Total dietary fibre**[*] mg/g plant extract	**Log colony forming units per mL (log_{10} CFU/mL) after 48 hours**[**+]		
		Bifidobacterium animalis[#]	*Lactobacillus rhamnosus*[##]	*Lactobacillus acidophilus*[#]
Inulin (control)		7.75 ±0.05[a]	8.58 ±0.01[a]	6.46 ±0.02[a]
Broth (baseline)		7.52 ±0.07	8.51 ±0.01	5.72 ±0.12
Rubus cuneifolius	21.24 ±0.38	7.84 ±0.06	8.45 ±0.03	6.63 ±0.04
Opuntia megacantha	20.95 ±0.12	7.72 ±0.12	8.56 ±0.02	6.10 ±0.05
Sonchus dregeanus	44.30 ±2.36	7.98 ±0.03[c]	8.40 ±0.02	6.20 ±0.03
Rorippa nudiuscula	37.00 ±5.17	8.15 ±0.05[d]	8.75 ±0.01[b]	6.48 ±0.04
Rosa rubiginosa	39.97 ±1.66	7.46 ±0.15	8.65 ±0.02	6.47 ±0.02
Amaranthus retroflexus	21.69 ±1.80	7.79 ±0.10	8.59 ±0.01	6.15 ±0.05
Chenopodium album	23.09 ±0.61	7.77 ±0.07	8.82 ±0.01[c]	5.59 ±0.11
Urtica dioica	34.68 ±4.88	8.01 ±0.06[c]	8.85 ±0.01[c]	0 (bactericidal)
Wahlengergia androsacea	32.21 ±0.53	7.84 ±0.06	8.57 ±0.02	6.41 ±0.06
Lepidium capense	14.74 ±1.04	7.73 ±0.05	8.72 ±0.01	6.86 ±0.01
Tragopogon porrifolius	21.78 ±1.44	7.59 ±0.11	8.73 ±0.01	7.05 ±0.01[b]
Sonchus oleraceus	19.25 ±1.69	7.42 ±0.10	8.58 ±0.04	6.87 ±0.04
Sonchus asper	19.96 ±0.92	7.66 ±0.10	8.79 ±0.01[b]	6.91 ±0.02[b]
Sisymbrium capense	7.97 ±1.35	7.56 ±0.07	8.48 ±0.04	5.76 ±0.15
Nemesia fruticans	22.59 ±2.72	7.95 ±0.05[b]	8.49 ±0.03	6.63 ±0.08
Nasturtium officinale	16.86 ±0.81	7.66 ±0.10	8.77 ±0.01	6.94 ±0.02[b]
Berkheya purpurea	31.21 ±2.13	7.77 ±0.07	8.64 ±0.02	6.62 ±0.03
Hypochaeris radicata	18.88 ±0.40	7.95 ±0.05[b]	8.53 ±0.01	6.65 ±0.04
Sisymbrium thelungii	41.77 ±2.34	8.02 ±0.06[c]	8.76 ±0.01[b]	6.78 ±0.04
Cyperus esculentus	36.13 ±0.95	7.84 ±0.06	8.66 ±0.01	6.83 ±0.03
Hypoxis hirsute	73.48 ±3.48	7.46 ±0.15	8.71 ±0.01	NT[$]
Sonchus integrifolius	27.52 ±1.01	7.98 ±0.03[c]	8.71 ±0.01	5.46 ±0.15
Solanum retroflexum	19.58 ±0.46	7.86 ±0.09	8.74 ±0.01[b]	6.81 ±0.02
Momordica foetida	25.56 ±0.19	7.59 ±0.11	8.63 ±0.01	6.82 ±0.02
Corchorus tridens	39.44 ±0.67	7.75 ±0.05	8.68 ±0.01	5.46 ±0.15
Amaranthus hybridus	34.79 ±0.33	7.77 ±0.07	8.29 ±0.03	6.62 ±0.04
Amaranthus spinosus	55.29 ±1.82	7.82 ±0.04	8.41 ±0.02	6.31 ±0.03
Bidens pilosa	39.83 ±1.25	7.46 ±0.15	8.65 ±0.01	6.75 ±0.03
Momordica involucrate	47.93 ±0.91	7.42 ±0.10	8.37 ±0.03	5.90 ±0.05
Dioscorea minutiflora	21.50 ±0.74	7.00 ±0.01	8.63 ±0.02	6.99 ±0.08[b]

Note. Values are mean ± sd, n=2[*] and n=3[**]. [+]No further growth was observed at 72 hours. [#]Conversion factor for colony forming units $=1\times10^5$, *or* [##]1×10^3. [$]NT = Not tested (plant material was insufficient), Values with different superscript letters indicate significant statistical difference ($p<0.01$) in growth stimulation by the edible wild plants per probiotic strain.

Tragopogon porrifolius, *Sonchus asper*, *Nasturtium officinale*, *Berkheya purpurea*, *Sisymbrium thelungii*, *Cyperus esculentus*, *Hypoxis hirsute*, *Sonchas integrifolius*, *Solanum retroflexum*, *Momordica foetida*, *Corchorus tridens*, *Bidens pilosa* and *Discorea minutiflora*.

Urtica dioica had the highest prebiotic response on *L. rhamnosus* of 7-fold more than inulin (positive control). The total dietary fibre content of *Urtica dioica* was high (3.5%) making it a better prebiotic candidate. However, *Urtica dioica* had inhibitory effects on *L. acidophilus*, which indicates selective and varied responses by the bacterial strains to the edible wild plant extracts. Sreenivas and Lele (2013) studied the prebiotic activity of gourd family vegetable fibres using *Lactobacillus fermentum*. They reported that gourd family vegetables had significant prebiotic ability. In the present study, *Momordica foetida* also stimulated the growth of probiotics. Petkova and Denev (2013) reported that *Sonchus oleraceus* had high content of both prebiotic fructooligosaccharrides and inulin. This explains the high growth stimulatory effects of all *Sonchus* species that we observed in this study.

3.5.3 Effects of Edible Wild Plant Extracts on the Proliferation of *L. Acidophilus* (ATCC 314)

The *L. acidophilus* bacteria cells were very tiny, non-spore forming gram-positive rods. The colonies were tiny greyish-white in colour and non-glistering. Twenty-five edible wild plants showed prebiotic activity on *L. acidophilus* compared to the broth (negative control, Table 4). Twenty plants (*Rubus Cuneifolius*, *Sonchus dregeanus*, *Wahlengergia androsacea*, *Lepidium capense*, *Tragopogon porrifolius*, *Sonchus asper*, *Nemesia fruticans*, *Nasturtium officinale*, *Berkheya purpurea*, *Taraxacum officinale*, *Sisymbrium thelungii*, *Cyperus esculentus*, *Hypoxis hirsute*, *Solanum retroflexum*, *Momordica foetida*, *Amaranthus hybridus*, *Bidens pilosa*,

Discorea minutiflora and *Sonchas integrifolius*) possessed much higher prebiotic activity than both positive control (inulin) and the negative control.

Tragopogon porrifolius species had the highest prebiotic activity with log (cfu/mL)=7.05±0.01 after 48 hours while *Sonchus integrifolius* and *Corchorus tridens* with log (cfu/mL)=5.46±0.15 had the lowest prebiotic activity among the group. *Tragopogon porrifolius* and *Discorea minutiflora* stimulated the growth of *L. acidophilus* by 5-fold more than inulin (positive control). *Tragopogon porrifolius* had high total dietary fibre content while *Discorea minutiflora* had a mixture of total dietary fibre and high indigestible protein content. Five edible wild plants (i.e. *Rorippa nudiuscula*, *Rosa rubiginosa*, *Amarathus spinosus*, *Opuntia megacantha* and *Sisymbrium capense*) had low prebiotic activity than inulin (positive control). Notably, *Urtica dioica* inhibited the growth of *L. acidophilus* possibly by selective bactericidal mechanisms confirming a previous report by Modarresi-Chahardehi, Ibrahim, Fariza-Sulaiman and Mousavi (2012).

4. Limitations of the Study

The simulated methods used to prepare the plant extracts possibly result in loss of ascorbic acid and therefore the actual ascorbic acid content was not reported. The effect of final dish preparation methods especially boiling and cooking of edible wild plant material needs to be investigated. The DPPH method determined the total antioxidant activity related to many free radicals or reactive oxygen species. The antioxidant capacity reported herein is therefore a compound value, which cannot be attributed to one type of molecule although it was mostly correlated to ascorbic acid content. The individual composition of other antioxidants in these edible wild plants may be determined prior to *in vivo* studies.

5. Conclusion

Eighty seven percent (87%) (n=30) of the edible wild plants met the FDA recommended daily value for ascorbic acid. *Cyperus esculentus* and *Rosa rubiginosa* had the highest content of ascorbic acid and correspondingly highly potent antioxidant activities while the Sonchus family consistently showed high ascorbic acid content and potent antioxidant activity. The antioxidant capacity of edible wild plants partly depends on their ascorbic acid content. Antioxidant activity observed for plant extracts with low ascorbic acid content suggest the presence of other types of antioxidants in the edible wild plants. *Rubus Cuneifolius*, *Lepidium capense*, *Berkheya purpurea*, *Sisymbrium thelungii*, *Cyperus esculentus*, and *Solanum retroflexum* stimulated the growth of all the three probiotics strains. Other edible wild plants demonstrated prebiotic activity on at least two of the probiotics while those that stimulated the growth of *B. animalis* and *L. rhamnosus* did not necessarily show the same trends with *L. acidophilus*. Probiotics may have selective nutritional preferences and different sensitivities to non-prebiotic phytochemicals in certain edible wild plants possibly due to different metabolic disposition of both the probiotics and the prebiotics.

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Conflict of Interest Statement

The authors hereby declare that they have no conflicts of interest.

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