

# Total Phenolic Compounds, Carotenoids and *In Vitro* Antioxidant Activity of Three Traditional Indigenous Medicinal Plants of Saskatchewan, Canada

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## Abstract

Medicinal plants are an important component in Indigenous cultures. *Aralia nudicaulis* L., *Rubus idaeus* L., and *Rosa arkansana* Porter were analyzed for total phenolic compounds, carotenoids and antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric-reducing antioxidant power), and ABTS (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid). The samples were harvested in Saskatchewan, Canada, with the help of an Indigenous Traditional Knowledge Keeper and the analyses were performed by spectrophotometry. The results showed that total phenolic compounds amount ranged from 0.08 to 0.88 mg GAE/mg d.w. and the total carotenoid contents ranged from 0.03 to 1.26 mg/g d.w. The *in vitro* antioxidant activity ranged from 0.02 to 0.71  $\mu\text{mol TE/mg d.w.}$  by DPPH, from 0.05 to 2.53  $\mu\text{mol TE/mg d.w.}$  by FRAP, and from 0.04 to 1.06  $\mu\text{mol TE/mg d.w.}$  by ABTS. *Rosa arkansana* leaves stood out with higher amounts of total phenolic compounds (TPC) ( $0.88 \pm 0.02$  mg GAE/g d.w.), carotenoids (TC) ( $1.26 \pm 0.03$  mg/g d.w.) and antioxidant activity (TAA) by DPPH ( $0.71 \pm 0.01$   $\mu\text{mol TE/mg d.w.}$ ), ABTS ( $1.06 \pm 0.01$   $\mu\text{mol TE/mg d.w.}$ ) and FRAP ( $2.32 \pm 0.01$   $\mu\text{mol TE/mg d.w.}$ ), with the same amount of *Rubus idaeus* belowground ( $2.53 \pm 0.01$   $\mu\text{mol TE/mg d.w.}$ ) in last technique ( $2.32 \pm 0.01$   $\mu\text{mol TE/mg d.w.}$ ). The first principal component describes 83.88% of the total variance and all the variables have high influence on this component (factor loadings: T = 0.976, TC = 0.735, TAA by DPPH = 0.955, FRAP = 0.894 and ABTS = 0.996), demonstrating that these samples do not have large dissimilarity. The second principal component represents 13.64% of the total variance, and the TC is the

dominant variable on the second principal component (0.658). *Aralia nudicaulis*, *Rubus idaeus*, and *Rosa arkansana* had interesting amounts of total phenolic compounds, total carotenoids and *in vitro* antioxidant activity. *Rosa arkansana* leaves and *Rubus idaeus* have the highest amount of total phenolic compounds and antioxidant activity in this study. *Rosa arkansana* leaves are also a good source of carotenoids, and so, they have great potential health benefits and use in industry as a source of bioactive compounds with high antioxidant activity. This study enriches the literature on medicinal plants used by Indigenous people of Saskatchewan and surrounding Canada. More studies are necessary to identify its applications, security and to assess which compounds generate the benefits reported by Traditional Knowledge Keepers.

### Keywords

*Aralia nudicaulis* L., *Rubus idaeus* L., *Rosa arkansana* Porter, DPPH, FRAP, ABTS

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## 1. Introduction

The transmission of Indigenous Knowledge and skills is essential to maintain customs and culture of Indigenous communities. Cultural continuity is a determining factor for the health of Indigenous people in Canada and the United States [1]. One class of knowledge that Elders and Traditional Knowledge Keepers (TKK) transmit is the management and use of medicinal plants and foods to combat and prevent diseases. Wild sarsaparilla or rabbit root (*Aralia nudicaulis* L.), wild red raspberry (*Rubus idaeus* L.) and prairie rose or rose hips (*Rosa arkansana* Porter) are Canadian examples of these medicinal plants, and their uses, described by a TKK, are summarized in **Table 1**. Although they have long been important medicinal plants throughout Southern Saskatchewan, very little information is available on these plants, especially *Aralia nudicaulis* and *Rosa arkansana*.

*Aralia nudicaulis*, commonly known as wild sarsaparilla, rabbit root or “Rabbit Berry” by the Dene people is a perennial plant growing up to 60 cm high from a rhizome that extends to 30 - 40 cm into the ground. Its single compound leaf is divided into 3 groups of 3 to 5 leaflets. This member of the ginseng family of *Araliaceae* is found in parkland groves and wooded ravines of the prairie area in Saskatchewan [2]. *Rubus idaeus*, commonly known as red raspberry, belongs to the *Rosaceae* family and is a common shrub species growing up to 2 m and is largely cultivated for its berries, which are botanically described as clusters of small drupelets. Although its aggregate fruits are popular for eating in a variety of ways, its stems and roots can also be used to make a beverage [3] [4]. *Rubus idaeus*' prickly stems support alternate compound leaves divided into 3 - 5 leaflets. The shrub *Rosa arkansana*, commonly known as prairie rose, is another

**Table 1.** Uses description of three Canadian medicinal plants as shared by a Traditional Knowledge Keeper in July 2019.

Medicinal plants	Traditional uses
Wild sarsaparilla or rabbit root ( <i>Aralia nudicaulis</i> L.)	“Only the outer layer of the bark on the root is used. The root is chewed and it gives you energy. It is good for men. It is good for blood circulation and is used in heart mixture. It is a blood circulation medicine. Same family as ginseng. You can chop the root until you have a powder and drink as tea. You should dry the roots in the sun.”
Wild red raspberry ( <i>Rubus idaeus</i> L.)	“The roots are good for women. Women medicine. You chop the roots, grind them until you have a powder. Drink as tea. It cleans internal sexual organs for fertility.”
Prairie rose or rose hips ( <i>Rosa arkansana</i> Porter)	“The red and ripe berries are high in vitamin C. The bark is good for eye sickness. It will give you an ‘itchy ass’ if you eat the berries when they are not ripe. You dry the plant, peel the bark off and grind it until it looks like powder. Drink it as tea.”

North American member of the *Rosaceae* family. Its stems are covered with thorns and support alternate leaves divided into 5 to 11 leaflets. Its rose hips contain fruits called achenes. Rose hips from various species have medicinal properties and are commonly used across North America, Europe, and Middle East [5].

Hexane extracts from *Aralia nudicaulis* rhizomes and fruits were very potent in killing human colon cancer cells, leukemia cells, and cervix cancer cells [6]. This plant also presented antimycobacterial activity against *Mycobacterium tuberculosis*, the mycobacteria causing tuberculosis [7]. *Rubus idaeus* L. berries demonstrated significant general antioxidant, antimicrobial, and anti-inflammatory properties, as well as antiproliferative properties against cancer cells [8]. Some *Rosa* species have been proven effective in the treatment of cancer, rheumatoid arthritis, diabetes, and neurodegenerative diseases [5].

Few studies have examined the bioactive compounds present in *Aralia nudicaulis*. In terms of antioxidant activity, methanol extracts of *Aralia nudicaulis* roots showed peroxy radical scavenging activity similar to black tea [9]. Methanol extract of *Aralia racemosa* seeds, however, showed very low antioxidant level [10]. Common phenolic antioxidant compounds such as flavonoids, tannins, and anthocyanins were found in *Rubus idaeus*. This plant expresses higher quantities of total phenolic compounds and therefore higher antioxidant activity in its leaf extracts compared to its fruit extracts [11]. The antioxidant activity of *Rosa arkansana* is also linked to the presence of polyphenols, carotenoids, and vitamins B, C, and E [5].

Most of the health benefits of these plants come from their antioxidant activities, which make them potent agents against oxidative damage from free radicals [12] [13]. Bioactive compounds, such as phenols and carotenoids, are largely responsible for the antioxidant activity of these plants [5]. Traditional medicinal plants may have higher bioactive compound content and antioxidant activity

than traditional foods and beverages, and are therefore of interest to food and pharmaceutical industries.

## 2. Objective

The objective of this study was to quantify the total antioxidant activity, and total phenolic and carotenoid compound content of *Aralia nudicaulis*, *Rubus idaeus*, and *Rosa arkansana* by three different analytical methods.

## 3. Materials and Methods

### 3.1. Plant Materials

*Aralia nudicaulis* L., *Rubus idaeus* L., and *Rosa arkansana* Porter were harvested in July 2019 during a medicine walk with a TKK in Piapot First Nation (49°47'32"N, 104°51'47"W), Saskatchewan, Canada. Before the medicinal plants could be harvested, proper Indigenous protocol was followed in the form of offering tobacco to the TKK in exchange for his knowledge and advice about which plants to select during the Medicine Walk. Traditional use of these plants shared by the TKK is reported in **Table 1**. After cleaning with water and drying in air at room temperature for 2 weeks, *Aralia nudicaulis* and *Rubus idaeus* were separated into aboveground and belowground parts and *Rosa arkansana* was separated into stems and leaves. The material was sealed and stored at  $-80^{\circ}\text{C}$ .

### 3.2. Chemicals and Instrumentation

Deionized water was used to prepare all chemical reagents. Glacial acetic acid was used to prepare the extraction solvent. Folin Ciocalteu reagent was obtained from Millipore Sigma. Anhydrous gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were obtained from Thermo Fisher Scientific. 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from TCI America (division of TCI, formerly Tokyo Chemical Industry). HPLC (High Performance Liquid Chromatography) grade solvents were used in chemical assays and extractions. The absorbance was measured on a Cary 100 Bio UV-Vis spectrophotometer. The 2.8 L VWR sonicator/ultrasonic cleaner was used in plant extractions.

### 3.3. Preparation of Reagents

The 10% Folin-Ciocalteu reagent was prepared by diluting the stock solution in deionized water. The DPPH reagent was prepared by dissolving DPPH in ethanol to a concentration of  $2 \times 10^{-4}$  g/mL. The FRAP's (ferric reducing ability of plasma or ferric ion reducing antioxidant power) reagent was prepared by dissolving 10% v/v 10 mM TPTZ solution (in 40 mM HCl) and 10% v/v 20 mM  $\text{FeCl}_3$  solution in the 0.3 M acetate buffer (pH 3.6).

The ABTS's reagent was prepared by mixing 88  $\mu\text{L}$  of 140 mM potassium persulphate solution with 5 mL of ABTS's 7 mM stock solution and the solution

was kept in darkness at room temperature. After 16 h, 1 mL of this solution was diluted in approximately 100 mL of ethanol and calibrated to the absorbance of  $0.7 \pm 0.05$  at 734 nm.

### 3.4. Total Phenolic Compounds and Antioxidants Extraction

The extraction was carried out using acidic methanol as described by Borges *et al.* [14] with modification. 25 mg plant material was extracted with 5 mL methanol:water:acetic acid (80:19:1, v:v); the mixture was vortexed followed by sonication for 30 min. After centrifugation for 10 min at 6000 rpm, the supernatant was removed and stored in amber glass. This extraction process was repeated to the pellet, resulting in 10 mL of extract.

### 3.5. Total Phenolic Compounds (TPC) Determination

TPC was determined using the Folin-Ciocalteu reagent, as described by Singleton & Rossi [15]. 0.5 mL of the extract was vortexed with 0.5 mL of deionized water, 0.5 mL of 10% Folin-Ciocalteu solution, and 2.5 mL of 4%  $\text{Na}_2\text{CO}_3$  solution. After kept in darkness for 1 h, the absorbance of the solution at 725 nm was measured and the TPC concentration was determined with a gallic acid standard curve and the concentration was expressed in mg gallic acid equivalents (GAE)/g d.w. (dry weight).

### 3.6. Total Antioxidant Activity (TAA) Determination

TAA was measured using three different methods: DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric-reducing antioxidant power) and ABTS (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)).

#### *DPPH*

The DPPH assay was performed as described by Brand-Williams *et al.* [16] with modification. 0.5 mL of the extract, 3 mL of ethanol P.A. and 0.3 mL of DPPH solution were vortexed. After keeping in darkness for 1 h, the absorbance at 517 nm was measured. The TAA concentration was determined with a Trolox standard curve and the concentration was expressed in  $\mu\text{mol}$  trolox equivalents (TE)/mg d.w.

#### *FRAP*

The FRAP assay was performed as described by Benzie & Strain [17] with modification. 30  $\mu\text{L}$  of the extract, 90  $\mu\text{L}$  of deionized water, and 900  $\mu\text{L}$  of FRAP's reagent were vortexed. After keeping in darkness for 1 h, the absorbance at 594 nm was measured. The TAA concentration was determined with a Trolox standard curve and the concentration was expressed in  $\mu\text{mol}$  trolox equivalents (TE)/mg d.w.

#### *ABTS*

The ABTS assay was performed as described by Re *et al.* [18] with modification. 30  $\mu\text{L}$  of the extract and 3 mL of ABTS's solution were vortexed. After keeping in darkness for 6 min, the absorbance at 734 nm was measured. The

TAA was determined with a trolox standard curve and the concentration was expressed in  $\mu\text{mol}$  trolox equivalents (TE)/mg d.w.

### 3.7. Total Carotenoids Determination

Total carotenoids (TC) analysis was performed as described by Lichtentaler [19]. 25 mg of plant material was mixed with 5 mL of 80% v/v acetone solution and the mixture was vortexed followed by sonication for 30 min. After centrifugation for 10 min at 6000 rpm, the absorbance of the supernatant was measured at 450 nm, 646 nm, and 663 nm respectively to determine the total carotenoids and the results were expressed in mg/g d.w.

### 3.8. Statistical Analysis

The total phenolic compounds, total carotenoids, and total antioxidant activity obtained by DPPH, FRAP, and ABTS were submitted to variance analysis (ANOVA), followed by Scott Knott ( $p < 0.05$ ) averages comparison test using the SISVAR program. Pearson's correlation ( $p < 0.05$ ) (SAS 9.1.) and principal component analysis (PCA) were performed to total phenolic compounds, total carotenoids, and total antioxidant activity obtained by DPPH, FRAP, and ABTS of the three plants studied here by using the software XLSTAT Version 2014.2.03 (STATCON, Witzenhausen, Germany).

## 4. Results and Discussion

In the traditional medicinal plants from this study, the contents of TPC ranged from 0.04 to 0.88 mg GAE/g d.w., the total carotenoid contents ranged from 0.03 to 1.26 mg/g d.w., and the TAA ranged from 0.02 to 0.69  $\mu\text{mol}$  TE/mg d.w. by DPPH, from 0.05 to 2.44  $\mu\text{mol}$  TE/mg d.w. by FRAP and from 0.04 to 1.02  $\mu\text{mol}$  TE/mg d.w. by ABTS (Table 2).

The content of TPC found in this study (0.04 - 0.88 mg GAE/g d.w.) was lower than what was found in known East Central Europe medicinal and industrial crop plants of 26 species of families *Asteraceae*, *Rosaceae* and *Lamiaceae* (0.8 -

**Table 2.** Total phenolic compounds, total carotenoids and total antioxidant activity by DPPH, FRAP and ABTS in different prairie medicinal plants.

Medicinal plant		TPC (mg GAE/g d.w.)	TC (mg/g d.w.)	DPPH ( $\mu\text{mol}$ TE/mg d.w.)	FRAP ( $\mu\text{mol}$ TE/mg d.w.)	ABTS ( $\mu\text{mol}$ TE/mg d.w.)
<i>Aralia nudicaulis</i>	aboveground	0.20 $\pm$ <0.01 <sup>d</sup>	0.73 $\pm$ 0.06 <sup>b</sup>	0.04 $\pm$ <0.01 <sup>d</sup>	0.17 $\pm$ <0.01 <sup>c</sup>	0.11 $\pm$ <0.01 <sup>d</sup>
	belowground	0.09 $\pm$ <0.01 <sup>e</sup>	0.03 $\pm$ <0.01 <sup>e</sup>	0.02 $\pm$ <0.01 <sup>e</sup>	0.05 $\pm$ 0.01 <sup>c</sup>	0.04 $\pm$ <0.01 <sup>d</sup>
<i>Rubus idaeus</i>	aboveground	0.04 $\pm$ 0.02 <sup>c</sup>	0.47 $\pm$ 0.05 <sup>c</sup>	0.32 $\pm$ <0.01 <sup>b</sup>	0.51 $\pm$ 0.01 <sup>b</sup>	0.37 $\pm$ <0.01 <sup>c</sup>
	belowground	0.74 $\pm$ 0.02 <sup>b</sup>	0.14 $\pm$ <0.01 <sup>d</sup>	0.25 $\pm$ <0.01 <sup>c</sup>	2.44 $\pm$ 0.01 <sup>a</sup>	0.63 $\pm$ <0.01 <sup>b</sup>
<i>Rosa arkansana</i>	stems	0.08 $\pm$ 0.01 <sup>e</sup>	0.05 $\pm$ 0.01 <sup>e</sup>	0.05 $\pm$ <0.01 <sup>d</sup>	0.07 $\pm$ 0.01 <sup>c</sup>	0.06 $\pm$ <0.01 <sup>d</sup>
	leaves	0.88 $\pm$ 0.02 <sup>a</sup>	1.26 $\pm$ 0.03 <sup>a</sup>	0.69 $\pm$ <0.01 <sup>a</sup>	2.23 $\pm$ 0.01 <sup>a</sup>	1.02 $\pm$ <0.01 <sup>a</sup>

\* Equal letters do not differ by Scott Knott test ( $p = 0.05$ ). TPC: Total Phenolic Compounds; TC: Total Carotenoids; TAA: Total Antioxidant Activity; GAE: Gallic Acid Equivalents; TE: Trolox Equivalents; d.w.: dry weight. Average  $\pm$  Standard Deviation.

18.6 mg GAE/g d.w.) [20], but in the same range of 112 Chinese medicinal plants associated with anti-cancer activity (0.02 - 5.03 mg GAE/g d.w.). TAA of some medicinal plants studied here were higher than the amount found in Chinese medicinal plants (0.17 - 0.47  $\mu\text{mol TE/g d. w.}$ ) [21]. TC content was in the same range found in 20 medicinal plant species of traditional Malay midwifery postnatal bath (0.1 - 1.5 mg/g d.w.) [22].

Phenolic compounds in plants are involved in growth regulation, disease resistance, and stress resistance towards low temperatures and excess of ultraviolet radiation [5] [23]. There is interest in surveying the antioxidant activity of native plants [10] and our results indicated that the medicinal plant knowledge of North American Indigenous people can be a valid source of potential bioactive compounds.

Phenolic compounds have high antioxidant activity [24]. In the current study, this class of compounds showed a strong positive correlation with TAA by DPPH ( $r = 0.885$ ) and very strong with FRAP ( $r = 0.959$ ) and ABTS ( $r = 0.975$ ), which suggested that the phenolic compounds of these plants might be responsible for their significant antioxidant capacities.

Carotenoids also could show noteworthy antioxidant activity [25]. In the plants studied here, TC showed a strong positive correlation with antioxidant activity by DPPH ( $r = 0.765$ ) and moderate correlation with ABTS ( $r = 0.674$ ), indicating that these substances are also responsible for antioxidant activity in these samples, but with less potency than phenolic compound classes.

Free radicals are odd-electron atoms or molecules with an unpaired electron in their valence orbital, which makes them unstable and highly reactive. Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products. ROS play several physiological roles (*i.e.*, cell signaling), and they are normally generated as by-products of oxygen metabolism. Despite this, environmental stressors (*i.e.*, UV, ionizing radiations, pollutants, and heavy metals) and xenobiotics (*i.e.*, antiproliferative drugs) contribute to greatly increase ROS production, therefore causing the imbalance that leads to cell and tissue damage (oxidative stress) [26].

The oxidative stress harmful effects on cellular systems have been associated in the development of a variety of diseases such as neurodegeneration [12], liver disease [13], diabetes [9] and cancers. Cancer onset in humans is a complex process, which requires both cellular and molecular alterations mediated by endogenous and/or exogenous triggers. It is already well known that oxidative DNA damage is one of those stimuli responsible for cancer development [26]. Antioxidant defense systems mitigate oxidative damage from free radicals and it has been shown that antioxidants have a protective role and can be used for the prevention and treatment of these diseases [12] [13].

Among our samples, *Rosa arkansana* leaves stood out with higher amounts of TPC ( $0.88 \pm 0.02$  mg GAE/g d.w.), TC ( $1.26 \pm 0.03$  mg/g d.w.), TAA by DPPH ( $0.71 \pm 0.01$   $\mu\text{mol TE/mg d.w.}$ ), ABTS ( $1.06 \pm 0.01$   $\mu\text{mol TE/mg d.w.}$ ), and the

same amount of *Rubus idaeus* belowground ( $2.53 \pm 0.01 \mu\text{mol TE/mg d.w.}$ ) in FRAP analysis ( $2.32 \pm 0.01 \mu\text{mol TE/mg d.w.}$ ) (**Table 2**).

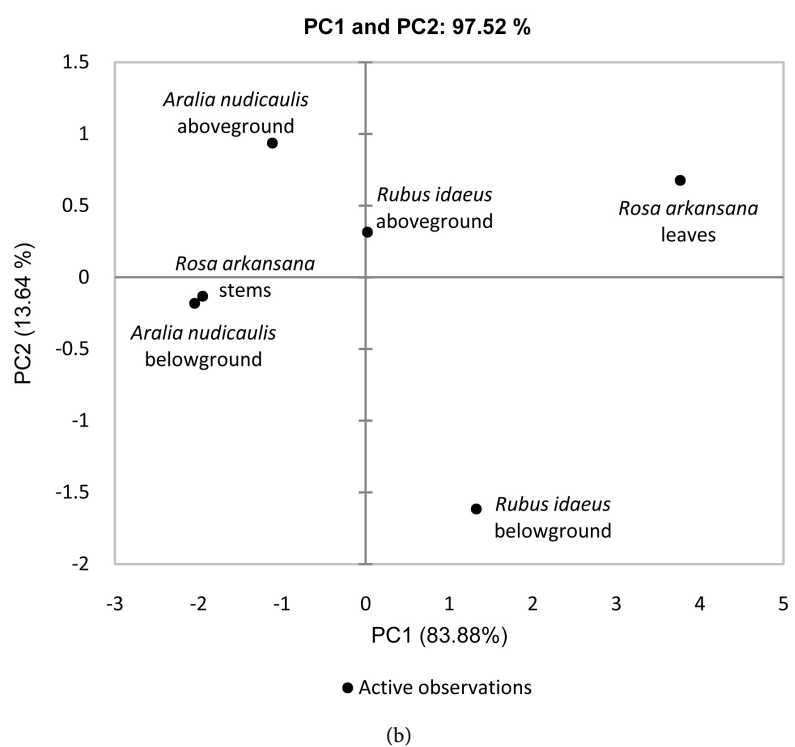
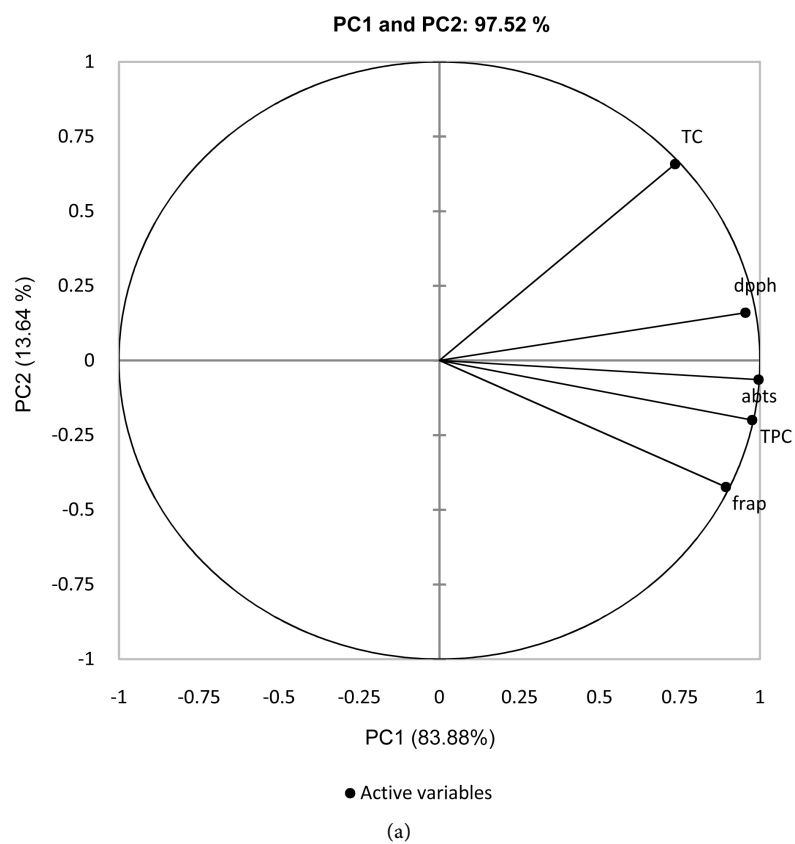
Most important phenolic acids present in *Rosa arkansana* are gallic acid, ellagic acid, syringic acid, vanillic acid, catechin, quercetin, kaempferol, myricetin, and rutin [5]. Caffeic acid, chlorogenic acid, *p*-coumaric acid, apigenin, and cinnamic acid were also found in varying concentrations in the petals of six *Rosa* species and these were related to their antioxidant activity [27]. More studies are needed to identify which are the phenolic compounds of the specific plants studied here.

According to the TKK, *Rosa arkansana* is recommended for eye sickness. The reddish color of its hips is produced by lipid-soluble carotenoids. A significant amount of total carotenoids were found in this plant in our study. This class of compounds can act as provitamin A compounds and help in the treatment and/or prevention of eye diseases [28]. More studies, however, are necessary to identify the specific carotenoids of *Rosa arkansana* and confirm if they actually have provitamin A activity.

*Rosa arkansana* fruits are known to have great quantities of bioactive compounds and antioxidant activity [29], and the present study showed that the leaves of this plant also have a good potential as a source of these compounds. However, further studies are needed to assess the safety of using this part of the plant in the food and pharmaceutical industry.

In this study, TPC, TC, and TAA differed significantly between aboveground and belowground plant material. Various phenolic compounds such as ellagic acid, polyphenols and flavonoids have been found in comparable concentrations in *Rubus idaeus* shoots, leaves, and fruits [4]. A linear relationship between total phenolic content and antioxidant activity in leaves has previously been reported [30]. The aboveground samples in the current study had shoots and leaves but no fruits were present. The former finding suggests that all aboveground components contain comparable phenolic compounds. Another study growing red raspberry cultivars, however, found that leaves have higher antioxidant activity than fruits, prompting the authors to suggest that antioxidant activity is specific to tissue type [30]. Its fruits, leaves, shoots, and roots are all traditionally used by Indigenous people in Canada to treat fevers, eye soreness, stomachache, and skin infections and strengthen women after giving birth [3]. Following harvest, the plant parts are usually allowed to dry and stored for later consumption. This practice is supported by the finding that dry shoot extract proved to be richer in phenolic compounds and this could be linked to higher antioxidant activity [4]. Factors such as the level of ripeness, manipulation techniques, geographical location and ecological factors all influence phenolic compound concentrations in *Rosa* hips [5].

An exploratory evaluation involving the three Canadian medicinal plants (aboveground and belowground) was performed using principal component analysis (PCA) for the bioactive compounds and antioxidant activity (**Figure 1(a)** and **Figure 1(b)**). Components (PC1  $\times$  PC2) described 97.52% of the total



**Figure 1.** Two-dimensional projection (a) and scores (b) from total phenolic compounds, carotenoids and antioxidant activity by three different methods (DPPH, FRAP and ABTS) in the two first principal components among different prairie medicinal plants. \*TPC: Total Phenolic Compounds, TC: Total Carotenoids.

variance of the data and provided discriminatory information related to the samples. **Figure 1(a)** showed the score plots (PC1 × PC2) of the PCA in relation to the TPC (mg GAE/g d.w.), TC (mg/g d.w.) and TAA by DPPH, ABTS and FRAP (μmol TE/mg d.w.).

The eigenvalues of the correlation matrix for PC1, PC2, PC3, PC4 and PC5 were, respectively: 4.194; 0.682; 0.116; 0.008 and <0.001.

The first principal component (PC1) described 83.88% of the total variance and all the variables had high influence on this component (factor loadings: TPC = 0.976, TC = 0.735, TAA by DPPH = 0.955, FRAP = 0.894 and ABTS = 0.996), demonstrating that these samples do not have large dissimilarity. The second principal component (PC2) represented 13.64% of the total variance, and the TC was the dominant variable on this PC (0.658). The other PCs represented just 2.48% of the total variance.

In **Figure 1(b)**, *Rosa arkansana* leaves and *Rubus idaeus* belowground parts were on the right side of the graph, while *Aralia nudicaulis* aboveground and belowground parts, and *Rosa arkansana* stems were on the left. *Rosa arkansana* leaves, *Aralia nudicaulis* aboveground parts and *Rubus idaeus* aboveground parts were in the upper quadrants of the graph, while *Rosa arkansana* stems, *Aralia nudicaulis* belowground parts and *Rubus idaeus* belowground parts were in the lower quadrants.

*Rosa arkansana* leaves and *Rubus idaeus* belowground parts had the highest TPC and TAA (DPPH and ABTS), which highlights and distances these samples from the others and their potential as a source of these compounds and as an antioxidant. *Rosa arkansana* leaves, *Aralia nudicaulis* aboveground parts and *Rubus idaeus* aboveground parts had similar behaviors and had higher TC content than stems and belowground parts. Leaves and aboveground parts may be more responsible for the pigments production of these plants.

*Rosa arkansana* leaves and *Rubus idaeus* belowground parts stood out in the right, showing that they had the highest positive correlation with amount of TPC and TAA in this study. The whole *Rubus idaeus* plant also showed a good potential as a source of these compounds and activity. *Rosa arkansana* leaves are a good source of TC too, and so, they have a great potential to health and industry as a source of bioactive compounds with high antioxidant activity. Further studies are necessary to identify its applications and safety.

Zhang *et al.* [31] studied the antioxidant and anti-inflammatory activities of 14 Chinese medicinal plants. Using DPPH assay, a strong correlation between total phenolic and flavonoid contents and the antioxidant activities were found. By Griess assay and ELISA, a considerable correlation between total phenolic and flavonoid contents and the anti-inflammatory activity was also found, suggesting the total phenolic and flavonoid contents play a role in anti-inflammatory potential. The work suggests that medicinal plants with high antioxidant activities from phenolic compounds have potential anti-inflammatory activities. Based on the high antioxidant activities observed in the three medicinal plants studied, we will look into the other bioactivities of these species. Further, it seems like the

simple antioxidant assays are powerful tool to screen the under-examined medicinal plants in the prairie for potential bioactivities.

## 5. Conclusion

*Aralia nudicaulis*, *Rubus idaeus*, and *Rosa arkansana* have interesting total phenolic and carotenoid compound contents and total antioxidant activity, therefore they have great potential for the pharmaceutical and food industry. Further studies are needed to assess the safety of these applications and to assess which compounds generate the benefits reported by the Traditional Knowledge Keeper.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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