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Short Communication

An Evaluation of Total Phenolic, Flavonoid and Tannin Contents of Three Herbs Used in the Treatment of Ailments in Kisii and Nyamira Counties, Kenya

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Abstract

Background and Aim: Herbs *Carissa spinarum*, *Physalis minima* and *Toddalia asiati* are used in the treatments of certain ailments in Kisii and Nyamira Counties region, Kenya but few studies have been conducted on the quality, efficacy and safety of using various parts of these herbs for these treatments. The principal aim of the present study was to standardize the three herbs of interest. This research was carried out to evaluate the total phenolic, flavonoid and tannin contents in the methanolic extract of whole plant *Physalis minima*, and leaves as wells roots of *Carissa spinarum* and *Toddalia asiatica*.

Materials and Methods: Each part of the herb was extracted by soaking in methanol/dichloromethane in ratio 1:1for seven days. Subsequently, it was filtered and concentrated by rotary vapor, and then the solvent allowed to escape and be solidified. The same process was repeated three times in all samples. Determination of the total phenolic content (mgGAE/g) was done by Folin-Ciocalteu on 765 nm using a spectrophotometer. The total flavonoid content (mgQE/g) was determined by aluminium chloride colorimetric assay on 415 nm. Total tannin content (mgCE/g) was analyzed by modified vanillin assay.

Results: The highest and lowest levels of total phenolic content were observed in the roots of *Toddalia asiatica* and *Carissa spinarum* respectively. The highest and lowest levels of total flavonoid content were observed in *Physalis minima* and in the root of *Carissa spinarum* respectively. The highest and lowest levels of total tannin content were observed in the leaf extract of *Toddalia asiatica* and root extract of *Carissa spinarum* respectively.

Conclusion: The highest total phenolic content was observed in the root extract of *Toddalia asiatica*. The highest total flavonoid content was observed in the extract of whole plant *Physalis minima*,while the highest total tannin content was observed in the leaf extract of *Toddalia asiatica*.

Keywords: Total content, Evaluation, Phenolic, Flavonoid, Tannin, Three herbs

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Introduction

The herbs *Carissa spinarum*, *Physalis minima* and *Toddalia asiatica* are used in the treatments of various ailments in Kisii and Nyamira counties region, Kenya but few studies have been conducted on the quality, efficacy and safety of using various parts of these herbs for these treatments. Characterized by better cultural acceptability, compatibility with the human body and minimal side effects, herbal medicine is inexpensive (1). *Carissa spinarum* has been used in the treatment of microbial infections such as venereal, respiratory and gastrointestinal infections (2). *Carissa spinarum* roots have been used in the treatment of inflammation disorders and snake bite (3). In Chinese system of medicine, *Carissa spinarum* has been used in the treatment of chest complaints, gonorrhoea, syphilis, rabies and sickle cell anaemia (4). *Carissa spinarum* extract is capable of having antibacterial and antioxidant activities (5). *Physalis minima* are useful in inflammations, antigonorrhoeic, enlargement of the spleen and abdominal troubles (6, 7). *Physalis minima* fruits and flowers could be utilized against stomach pain and constipation. Moreover, the herb paste is used in ear disorders, and ripen fruits are used in gastric trouble. *Physalis minima* decoction of the whole plant is consumed as a remedy for cancer. *Physalis minima* is used in the treatment of asthma, bronchitis, inflammation, enlargement of spleen, urinary disorder, abdominal troubles and headache while its roots are used in the treatment of diabetes (7). *Toddalia asitica* has antitumor, antibacterial, antifungal, anti-malaria and antiviral activities (8, 9). *Toddalia asitica* alkaloid in crude extract has anti-inflammatory, anti-malarial and anti-leukimatic properties (9). *Toddalia asitica* coumarine derivatives have antiplasmodial, antimicrobial activity and root decoctions are drunk to treat malaria (10). Herbal antioxidants possess multiple mechanisms of actions, and are non-toxic; therefore more acceptable than synthetic ones (11). Antioxidants are secondary metabolites that prevent damages to cells caused by free radicals, have the ability to repair previous damages to cells and search for free radicals, and lend them electron which stabilizes the molecule. Hence, they prevent damages to other cells (12**)**. Antioxidants are able to alter free radicals into waste by products, and they are finally removed from the body (12). Free radicals are associated with several diseases such as cancer, diabetes mellitus, arthritis and ageing which is treated by antioxidant therapy (12). The identification of antioxidant activities and total contents of phenolics, flavonoids and tannins in *Carissa spinarum*, *Physalis minima* and *Toddalia asiatica* extracts was due to the fact that antioxidants can prevent free radicals, mainly highly reactive oxygen and nitrogen species, from damaging human health (13). Free radicals are central to the development of tissue harm in several human diseases such as cancer, aging, neurodegenerative disease, malaria and arteriosclerosis, and pathological complications in living organisms (14). Antioxidants can have a significant role in the prevention of these diseases (14). The phenolic and flavonoid compounds of the herb extracts account for the antioxidant effect. The antioxidant feature of phenolics originates from their ability to donate an electron or hydrogen from phenolic hydroxyl groups (15). The resultant phenoxy radical is not highly reactive due to electron delocalization in the aromatic ring, hence reactive radical is replaced by those of limited activity (15). Polyphenols are bioactive compounds with various activities such as antioxidant, antiinflammatory, anticancer, anti-diabetic and anti-microbial properties (16, 17). Flavonoids are polyphenols that are effective in the management of diabetes and associated complications, (17, 18). Flavonoids are a significant class of anticancer therapeutic agents (18). They prevent the development of cancer by their antioxidant and immunomodulatory activities (19). Tannins are polyphenols that can donate hydrogen and act as antioxidants (20). Tannins have a crucial role in the treatment of many diseases (20). It has been indicated that tannis can prevent the development of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them. The growth of many fungi, yeasts, bacteria and viruses was hindered by tannins (21). The aim of this research was to examine the total phenolic, flavonoid and

tannin contents in the extract of whole plant *Physalis minima*, and leaf as well as root extract of *Carissa spinarum* and *Toddalia asiatica* used in the treatment of ailments in Kisii and Nyamira Counties region, Kenya.

Materials and Methods

This subsection lists the chemicals, equipment and glass wares to be used in conducting different experiments.

Chemicals

All the chemicals and reagents used were of analytical grade. They include the following: gallic acid, foline-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, quercetin, aluminium chloride, silica gel, sodium acetate, sodium carbonate, ethanol , HPL methanol, HPL hexane, TLC plates, Conc.H_2SO_4, Conc. HCl, C_2H_5OH, C_3H_6O, CH_2Cl_2, $CHCl_3$, NH_3, KI, nutrient agar, glacial acetic acid, olive oil, $C_2H_5OO^-$, NaOH, , dragencloffs reagent, 2% H_2S0_4, $(C_2H_5OO)_2Pb$, 1% KOH, 1M HCl , 1M H_2SO_4, 50% HNO_3, I_2, and 5%FCl_3, among other laboratory reagents.

Equipment

The equipment used include the following: Bruch Rota Vapours R-4000, (Rotary evaporator), UV-spectrophotometer, and several other laboratory equipment.

Plant Materials

Herb Identification and Collection

Parts of *Carrisa spinarum*, *Physalis minima* and *Toddalia asiatica* were collected in January 2015 from the same ecological zones in Kisii and Nyamira Counties region, Kenya. Verification of the herbs was carried out by Prof. S. M. Kariuki, the Botanist; Egerton University.

Preparation of Samples

Extraction of each selected herb of interest was carried out using standard procedures as described in certain references (22-25). The herb materials of each sample were collected and cleaned, dried at room temperature, crushed into powder and stored in an air tight glass container. The 2.3Kg powder leaf of *Toddalia asiatica* was weighed by electrical chemical balance, then soaked in 1:1 methanol in dichloromethane for five days to allow the phytochemical compounds to be extracted by the solvent. The same procedure for the leaf of *Toddalia asiatica* was repeated three times. The same process was also repeated for 1.45Kg root of *Toddalia asiatica*, 2.4721Kg leaf of *Carissa spinarum*, 1.2231 Kg root of *Carissa spinarum* and 1.144Kg of whole plant *Physalis minima*. The extracts of each sample were filtered by clean cotton wool and whatman filter paper No. 1, concentrated using a rotary vapor at 45°C. The concentrated extract was placed in the laboratory to allow the solvent escape and solidify awaiting further investigations.

Determination of Total Phenolic, Flavonoid and Tannin Contents

The total antioxidant content in each methanolic extract of whole plant *Physalis minima*, leaf and root of *Carissa spinarum*, and *Toddalia asiatica* was determined as described in the references (26, 27).

Determination of Total Phenolic Content

The total phenolic content in each extract of whole plant *Physalis minima*, leaf and root of *Carissa spinarum* and *Toddalia asiatica* was determined by UV-Visible Spectrophotometer (Shimadzu: UV-1601 PC) using the Folin-Ciocalteu method (26, 27). Gallic acid was used as the standard. 1.0 ml of each extract was mixed with 1.0 ml gallic acid reagent in methanol and 7.5% sodium carbonate and then allowed to stand for 40 minutes at room temperature. Absorbance was read at 765nm. The phenolic content in the methanolic extract was obtained from the standard curve, and was shown as gallic acid equivalent (GAE) in mg per gram dry weight (mg GAE/g) of the extracted compound. All the tests were done in triplicates.

Determination of Total Flavonoid Content

The determination of total flavonoid content was carried out as described in some references (27-30). The flavonoid content in the methanolic extracts of whole plant *Physalis minima*, leaf and root of *Carissa spinarum* and *Toddalia asiatica* was determined using aluminum chloride colorimetric assay (14, 15). Quercetin was used as the standard. 1ml of each extract solution was mixed with 1ml standard and added to methanol, sodium nitrite, and aluminum chloride. The mixture was incubated and added to NaOH. The mixture was allowed to cool, and the absorbance was measured at 510 nm with a spectrophotometer. The total flavonoid content was evaluated from the calibration curve and expressed as

mg quercetin equivalent per g of dry weight (mgQE/g) of the extracted compound. All the samples were tested in triplicates.

Determination of Total Content Tannin

The total tannin content was determined according to Nickavar *et al*. (30). The condensed tannin content was examined in detail by the modified vanillin assay. 1 ml of 2% aluminium chloride and 3 ml of 5% sodium acetate were added to 1 ml of each extract. The absorbance of each sample was measured after 2.5 hour at 440 nm with a spectrophotometer. The total tannin content was evaluated from the calibration curve and expressed as mg of catechin equivalents (CE) per gram of dry weight (mgCE/g) of the extracted compound.

Statistical Analysis

The total phenolic, flavonoid and tannin contents tests were determined in triplicate and the results were indicated as mean values ± standard deviation.

Results and Discussion

The results in table 1 show total phenolic, flavonoid and tannin contents in methanolic extracts, evaluated from regression equation of calibration curves.

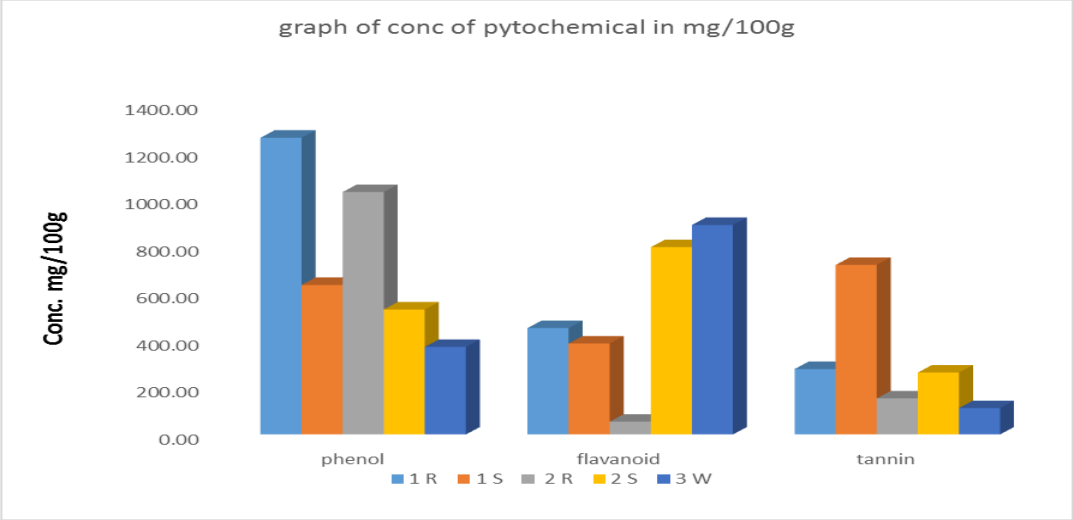
The Relationships between Total Phenolic and Flavonoid Contents and Antioxidation Activity Toddalia Asiatica

Total Phenolics Content

The results presented in Table 1 show the total phenolic content in the leaf and root extracts of *Toddalia asiatica* evaluated from the standard calibration curve. The calibration curve of gallic acid revealed maximum absorbances at 765 nm wave-length, (equation y = 0.0008x, R^2 = 0.997). The total phenolic content of the extract was reported as gallic acid equivalent and expressed as milligrams equivalent of gallic acid per gram of dry weight (mg GAE/g) of the extracted compound. The root extract of *Toddalia asiatica* showed higher total phenolic contents than their leaf extract (Figure 1 and Figure 2). It has been reported that the total phenolic content (mg GAE/g) of the ethanolic leaf and stem extracts of *Toddalia asiatica* were registered 0.0673 and 0.0407 compared to 6.364 and 12.60 in the methanolic leaf and root extracts of *Toddalia asiatica* obtained in this research (21). It has been reported that the highest total phenolic content was registered in the leaf extract of *Toddalia asiatica* compared to their root extract (20). In contrast, the results obtained in this research indicated that the root extracts of *Toddalia asiatica* had higher total phenolic contents than their leaf extract (Table 1 and Figure 2).

Total Flavonoids Content

The results presented in Table 1 indicate the total flavonoid content in the leaf and root extracts of *Toddalia asiatica* calculated from the standard



Key: 1R- *Toddalia asiatica* root**, 1S-** *Toddalia asiatica* leaf**, 2R -***Carissa spinarum* root**, 2S** *Carissa spinarum* leaf**, 3W-**whole plant **-***Physalis minima*

Figure 1. Phytochemical of three herbs from Kisii and Nyamira Counties, Kenya.

Table 1: Total Phenolic, Flavonoid and Tannin Contents: (Mean ± SD).

Solvent			**Methanol**		
			Phytochemical constituent		
Sample No.	Herbs	Plant part	**Total phenolic (mg GAE /g)**	**Total flavonoid (mgQE /g)**	**Total tannin (mgCE /g)**
1	*Carissa spinarum*	Leaf	5.2977 ± 3.767	7.9407 ± 3.831	2.6260 ± 8.107
		Root	10.2782 ± 1.755	0.5431 ± 0.00	1.5308 ± 9.358
2	*Physalis minima*	Whole plant	3.7114 ± 1.672	8.8831 ± 2.597	1.1170 ± 6.644
3	*Toddalia asiatica*	Leaf	6.3383 ± 2.539	3.8567 ± 1.491	7.1889 ± 18.108
		Root	12.5802 ± 2.43	4.5128 ± 6.54	2.7751 ± 14.55

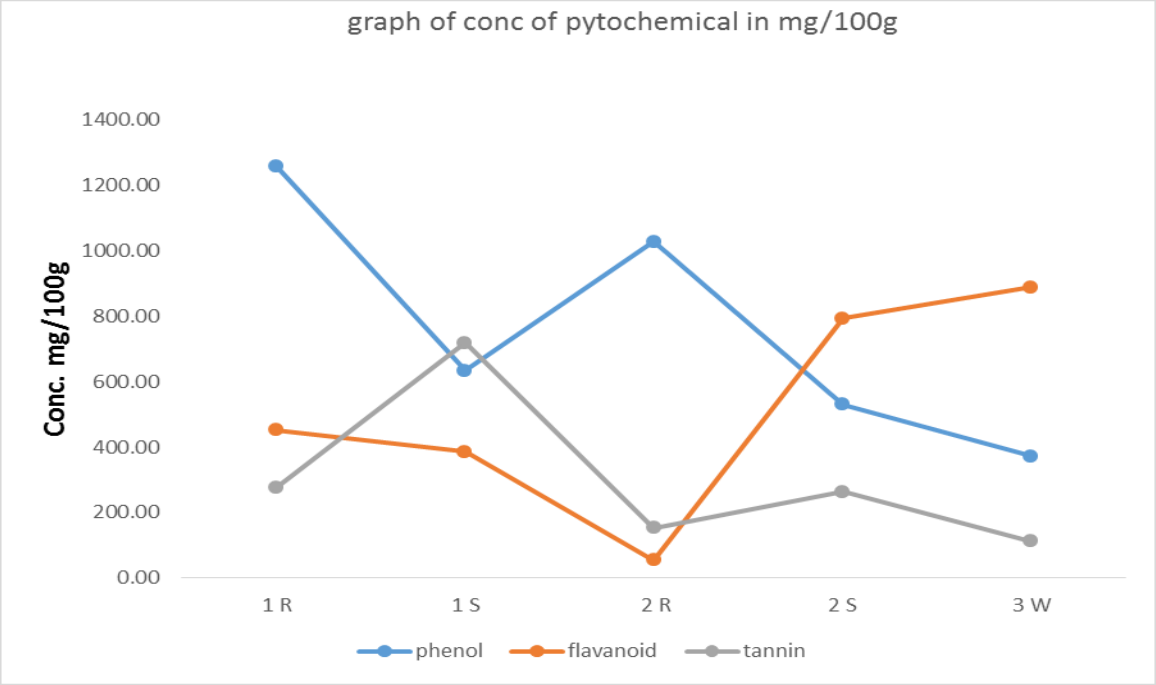
Table 2: Total Phenolic, Flavonoid Content and Antioxidant Activity of Three Herbs.

Sample	**Plant**	**plant Part**	**Total phenolic (mgGAE /g)**	**Total flavonoid (mgGAE/g)**	**DPPH scavenging activity (%)**	**IC_{50} (mg/ml)**
1	*Carissa spinarum*	Leaf	5.2977	7.9407	93.16	= 0.8
		Root	10.2782	0.5431	64.56	= 5
2	*Physalis minima*	Whole plant	3.7114	8.8831	84.27	= 1
3	*Toddalia asiatica*	Leaf	6.3383	3.8567	68.06	= 2
		Root	12.58	4.5128	30.75	Undetected
4	Ascorbic acid	STD	-	-	86.69	=1

calibration curve. The calibration curve of quercetin indicated maximum absorbance at 510 nm wave-length, (equation y = 0.0011x, R^2 = 0.9938). The total flavonoid content was calculated from the calibration curve and was expressed as mg quercetin equivalent per g of dry weight (mgQE/g) of the extracted compound. The root extract of *Toddalia asiatica* exhibited relatively higher total flavonoid contents than their leaf extract (Figure 1 and Figure 2). It has been reported that the total flavonoid content (mgQE/g) of the ethanolic leaf and stem extracts of *Toddalia asiatica* were registered 0.0821 and 0.0420 compared to 3.8716 and 4.5782 in the leaf and root extract of *Toddalia asiatica* obtained in this research (21).

Total Tannin Content

The results exhibited in Table 1 display the total tannin content in the leaf and root extracts of *Toddalia asiatica* evaluated from the standard calibration curve. The calibration curve of catechin indicated maximum absorbance at 765 nm wavelength, (equation y = 0.0008x, R^2 = 0.997) and was expressed as mg of catechin equivalents (CE) per gram of dry weight (mgCE/g) of the extracted compound. The leaf extract



Key: 1R- *Toddalia asiatica* root**, 1S-** *Toddalia asiatica* leaf**, 2R -***Carissa spinarum* root**, 2S** *Carissa spinarum* leaf**, 3W-**whole plant **-***Physalis minima*

Figure 2. Phytochemicals of Three Herbs Used in Kisii and Nyamira Counties region, Kenya.

of *Toddalia asiatica* exhibited higher total tannin contents than their root extract (Figure 1 and Figure 2). It was also reported that the total tannin content (mgCE /g) of the ethanolic leaf and stem extracts of *Toddalia asiatica* were recorded to be 0.0483 and 0.0152 compared to 7.27 and 2.9217 in the leaf and root extract of *Toddalia asiatica* obtained in this research (21).

Carissa spinarum

Total phenolics content

The results of Table 1 show the total phenolic content in the leaf and root extracts of *Carissa spinarum* evaluated from the standard calibration curve. The calibration curve of gallic acid revealed maximum absorbances at 765 nm wavelength, (equation y = 0.0008x, R^2 = 0.997). The total phenolic content of the extract was reported as gallic acid equivalents and expressed as milligrams equivalent of gallic acid per gram of dry weight (mgGAE/g) of the extracted compound. The root extract of *Carissa spinarum* revealed higher total phenolic contents than their leaf extract (Figures 1 and 2). It has been reported that the total phenolic content (mgGAE /g) of aqueous fruit extract of Carissa *spinarum* ranged from 5.90 ± 0.41 to 6.81 ± 0.02 compared to 5.2977 ± 3.767 and 10.2782 ± 1.755 of the methanolic leaf and root extracts of *Carissa spinarum* obtained in this research (31). It has been reported by (20) that a higher total phenolic content was registered in the leaf extract of *Carissa spinarum* than their root extract. In contrast, the results obtained in this research indicated that the root extract of *Carissa spinarum* had a higher total phenolic content than their leaf extract (Table 1 and Figure 2).

Total flavonoid content

The results of Table 1 indicate the total flavonoid content in the leaf and root extract of *Carissa spinarum* calculated from the standard calibration curve. The calibration curve of quercetin indicated maximum absorbance at 510 nm wavelength, (equation y = 0.0011x, R^2 = 0.9938). The total flavonoid content was calculated from the calibration curve and was expressed as mg quercetin equivalent per g of dry weight (mgQE/g) of the extracted compound. The leaf extract of *Carissa spinarum* had a higher total flavonoid content than their root extract

(Figure 1 and Figure 2). It has been reported by that the total flavonoid content (mgQE/g) of aqueous fruit extract of *Carissa spinarum* ranged from 5.95 ± 0.004 to 5.95 ± 0.76 compared to 7.9407 ± 3.831 and 5.431 ± 0.00 (mgQE/g) of the leaf and root extract of *Carissa spinarum* obtained in this research (31).

Total tannins content

The results presented in Table 1 display the total tannin content in the leaf and root extracts of *Carissa spinarum* calculated from the standard calibration curve. The calibration curve of catechin revealed maximum absorbance at 765 nm wavelength, (equation y = 0.0008x, R^2 = 0.997) and was expressed as mg of catechin equivalents (CE) per gram of dry weight (mgCE/g) of the extracted compound. The leaf extract of *Carissa spinarum* exhibited a relatively higher total tannin content than their root extract (Figure 1 and Figure 2).

Physalis Minima

Total Phenolics Content

The results of Table 1 reveal the total phenolic content in the extract of whole plant *Physalis minima* calculated from the standard calibration curve. The calibration curve of gallic acid showed maximum absorbances at 765 nm wavelength, (equation y = 0.0008x, R^2 = 0.997). The total phenolic content of the extract was reported as gallic acid equivalents and expressed as milligrams equivalent of gallic acid per gram of dry weight (mgGAE/g) of the extracted compound. The extract of whole plant *Physalis minima* displayed relatively high total phenolic contents (Figure 1 and Figure 2).

Total Flavonoid Content

The results presented in Table 1 show the total flavonoid content in the extract of whole plant *Physalis minima* calculated from the standard calibration curve. The calibration curve of quercetin disclosed maximum absorbance at 510 nm wavelength, (equation y = 0.0011x, R^2 = 0.9938). The total flavonoid content was calculated from the calibration curve and was expressed as mg quercetin equivalent per g of dry weight (mgQE/g) of the extracted compound. The extract of whole plant *Physalis minima* revealed high total flavonoid contents (Figure 1 and Figure 2).

Total Tannins Content

The results of Table 1 exhibit the total tannin content in the extract of whole plant *Physalis minima* evaluated from the standard calibration curve. The calibration curve of catechin showed maximum absorbance at 765 nm wavelength, (equation y = 0.0008x, R^2 = 0.997) and was expressed as mg of catechin equivalents (CE) per gram of dry weight (mgCE/g) of the extracted compound. The extract of whole plant *Physalis minima* displayed low total tannin contents (Figure 1 and Figure 2).

The Relationships between Total Phenolic and Flavonoid Contents and Antioxidation Activity

It has been reported that the existence of phenolic compounds and flavonoids increases the possibility of antioxidant activity, because it has been reported that these compounds have antioxidant properties (32). The results presented in Table 2 indicate that the leaf extract of *Carissa spinarum* exhibited the highest antioxidant activity compared to their root extracts, which might have been associated with relatively high phenolic and flavonoid contents. The whole plant extract of *Physalis minima* showed high antioxidant activity due to high flavonoid and phenolic contents. The leaf extract of *Toddalia asiatica* displayed higher antioxidant activity than their roots due to high phenolic and flavonoid contents. Phenols and flavonoids are produced as secondary metabolites in plant systems in order to protect them from oxidative stress. A strong and positive correlation has been reported between the phenolic content and the antioxidant activity of extracts which is in accordance with this research.

Conclusion

The existence of phenols, flavonoids and tannins in the extract of whole plant *Physalis minima*, *leaf* and root extracts of *Carissa spinarum* and *Toddalia sciatica* indicates that the traditional use of these herbs in the treatment of certain diseases is reasonable. Moreover, this phenomenon confirms the validity of their claim of being used for the aforementioned purpose in folklore medicine. An investigation of the total antioxidant content and free radicals scavenging activity of *Carrissa spinarum*, *Physalis minima* and *Toddalia asiatica* provided the scientific basis for the medicinal applications of these herbs and enhanced their exploitation in the management of diseases. This finding provided information which revealed that the

selected three herbs of interest might potentially have free radical scavenging, antibacterial and antifungal activities.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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