

## *In vitro* investigation of antioxidant activities of *Launea taraxacifolia* and *Crassocephalum rubens*

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### Abstract

*Launea taraxacifolia* and *Crassocephalum rubens* are among many wild, underutilized and under cultivated vegetables in Nigeria that are at risk of extinction. Total flavonoid contents (TFC), total phenolic contents (TPC), and antioxidant activities of different concentrations (1-5 mg ml<sup>-1</sup>) were evaluated; using *in vitro* assays to assess the scavenging properties of 2, 2-diphenyl-1-picryl hydrazyl (DPPHRSP), nitric oxide (NORSP) and hydroxyl (OHRSP). Phenolic profiles of the alcoholic extracts were characterized using high-performance liquid chromatography techniques. The results revealed higher TFC (mg/100g RE) in aqueous (6.06±0.02-78.79±0.01) than alcohol extracts (with methanol 0.93±0.01–12.73±0.04, and with ethanol -0.85±0.01–7.70±0.03). In a similar trend, OHRSP (%) was higher in aqueous extracts (40.83±0.10–91.74±0.19) than alcoholic extracts (with methanol -11.67±0.3–30.83±0.06; and with ethanol -14.42±0.06–40.27±0.05). TPC (mg/100g GAE) which was higher in alcoholic extracts (with methanol -21.48±0.01–133.20±0.16 and with ethanol -9.45±0.01–59.73±0.02) than aqueous extracts (14.83±0.01–52.64±0.03) was in agreement with the trend observed for NORSP (28.24±0.05–151.76±0.08 for methanolic extracts, 21.99±0.13–49.93±0.04 for ethanolic extracts and 38.47±0.11–86.15±0.05 for aqueous extracts). DPPHRSP was also higher in alcoholic extracts (methanolic -22.81±0.01–48.41±0.05 and ethanolic -14.53±0.01–62.68±0.07) than aqueous extracts (13.66±0.13–42.86±0.03). TFC, TPC and antioxidant activities showed concentration dependent increase and strong positive correlation with TFC (r= 0.926 – 0.997 and r= 0.432 – 1.000) and TPC (r= 0.825 – 0.999 and r= 0.473 – 0.994) for *L. taraxacifolia* and *C. rubens* respectively. Caffeic acid, chlorogenic acid, ellagic acid, quercetin and kaempferol were identified as major phenolic components in the extracts. The vegetables have high antioxidant potential for promoting good health; which could be attributed to the identified phytochemicals in them.

**Keywords:** Underutilized; Vegetables; Phenolic; Flavonoid; Antioxidant

## 1 Introduction

The antioxidant activity of fruits and vegetables is generally positively correlated with their content of polyphenols (Wang & Lin, 2000; Koffi, Sea, Dodehe, & Soro, 2010). Free radicals generated in living organism during various pro-

cesses can cause extensive oxidative damage to cells during metabolism, leading to aging, cancer, neurodegenerative disorders, liver cirrhosis, atherosclerosis, diabetes, cardiovascular and other chronic diseases (Halliwell, 2007; Liu et al., 2010). Compounds such as vitamin C and E, carotenoids and phenolics are natural antiox-

idants that can slow or prevent this oxidative damage of other molecules thereby reducing the risk of diseases. The most effective antioxidants seem to be flavonoids and phenolic compounds of many plant raw materials, particularly in fruits, vegetables, herbs and seeds as many studies have suggested (Kim, Tsao, Yang, & Cui, 2006; Adom & Liu, 2002). Their metal-chelating capabilities and radical-scavenging properties have enabled phenolic compounds to be thought of as effective free radical scavengers and inhibitors of lipid peroxidation (Aruoma, 1998; Aruoma et al., 1997; Liu et al., 2010; Geng, Ren, Liu, & X., 2012). Many wild plants exist in Nigeria but are underutilized probably due to inadequate knowledge of their health promoting potential and sometimes due to dietary habit.

*L. taraxacifolia* and *C. rubens* are among many underutilized wild vegetables in western Nigeria. While *Crassocephalum rubens* is used as a vegetable by few, especially among the aged and rural dwellers, *L. taraxacifolia* is not yet recognized as food and cannot be found in any market in Ondo State, the part of Nigeria where this work was carried out. *Launaea taraxacifolia*, also known as wild lettuce belongs to the family Asteraceae. The leaf is used as a vegetable in some parts of South-Western Nigeria in West Africa where it is called *efo yanrin* and research has established its high nutritional and medicinal values (Adebisi, 2004). It is mostly collected from the wild and only cultivated to a limited extent for home use and for local markets; whereas, it is known only as a weed in many parts of Nigeria. *L. taraxacifolia* is nutritionally rich in vitamins, minerals, proteins, essential fatty acids, fibre content and flavonoids (Adinortey et al., 2012; Dickson et al., 2012; Gbadamosi & Sulaiman, 2012). *Crassocephalum rubens* (Juss. Exjacq. S. Moore) belongs to the plant family Asteraceae. It is a wild, economic and medicinal plant that is now threatened by genetic erosion in the Nigerian ecosystem. The scarcity of the plant is due to indiscriminate collection from the wild and the difficulty in germinating the seeds (Gbadamosi & Sulaiman, 2012).

Many researchers have reported the nutritional values of *L. taraxacifolia* and *C. rubens*, with the aim of drawing attention to them, but there is a dearth of information on their antioxidant

potentials. The present study was aimed at investigating phenolic composition and antioxidant activities of the different concentrations of aqueous and alcoholic extracts of the leaves of the above mentioned vegetables.

## 2 Materials and Methods

### 2.1 Sample collection

*L. taraxacifolia* was obtained from a nearby bush in Akure and *C. rubens* was purchased from a local market also in Akure, Ondo State, in Nigeria. Approximately 500 g was obtained for each.

### 2.2 Preparation of Samples and extracts

The air-dried vegetable samples were ground and sieved to give a 40 mm mesh size powder. A bioactive extract of each powdered vegetable was obtained by weighing 20 g into clean and dried reagent bottles, separately adding 400 mL of distilled water, methanol and ethanol into each bottle and subjecting them to a cold maceration process for 24 h to obtain the aqueous extract and 72 h to obtain the alcoholic extracts. Extracts were brought to dryness with a rotary evaporator at a low temperature (45 °C) and were kept at 3 °C in the refrigerator while the analyses were carried out (Iwalewa et al., 2005).

### 2.3 Quantification of alcohol extracts by HPLC-DAD

Chromatographic analyses were carried out under gradient conditions using a C<sub>18</sub> column (4.6 mm × 250 mm) in reverse phase (HPLC-DAD with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system, by Shimadzu, Kyoto, Japan), packed with 5 μm diameter particles, where the mobile phase was water containing 1% acetic acid (A) and methanol (B), and the composition gradient was: 5% of B until 10 min and changed to obtain 20%, 30%, 50%, 60%, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, following the method described by da Silva et al. (2014) with slight

modifications. Each extract of *L. taraxacifolia* and *C. rubens* was analyzed at a concentration of 20 mg/mL. The samples and mobile phase were filtered through a 0.45  $\mu\text{m}$  membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. The flow rate was 0.6 mL/min, injection volume 50  $\mu\text{L}$  and the wavelengths were 270 for gallic acid, 278 nm for coumarin, 327 nm for chlorogenic acid and caffeic acid, and 365 nm for quercetin, quercitrin, kaempferol and rutin. Chromatography peaks were confirmed by comparing their retention time with those of reference standards and by DAD spectra (200 to 500 nm). Calibration curves were as follows for gallic acid:  $Y = 13480x + 1257.5$  ( $r = 0.9998$ ); coumarin:  $Y = 11983x + 1196.9$  ( $r = 0.9997$ ); chlorogenic acid:  $Y = 11786x + 1267.1$  ( $r = 0.9991$ ); caffeic acid:  $Y = 13048x + 1345.6$  ( $r = 0.9995$ ); rutin:  $Y = 12478x + 1194.9$  ( $r = 0.9997$ ), quercitrin:  $Y = 13641x + 1178.4$  ( $r = 0.9997$ ), kaempferol:  $Y = 11458x + 1269.4$  ( $r = 0.9998$ ) and quercetin:  $Y = 12783x + 1195.8$  ( $r = 0.9996$ ). All chromatography operations were carried out at ambient temperature and in triplicate.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as  $3.3$  and  $10 \sigma/S$ , respectively, where  $\sigma$  is the standard deviation of the response and  $S$  is the slope of the calibration curve (da Silva et al., 2014).

## 2.4 Determination of total phenol contents

The total phenol content (TPC) of the samples was determined by mixing 0.5 mL of each extract ( $1\text{-}5\text{ mg mL}^{-1}$ ) with 2.5 mL 10 % Folin-Cioaltea's reagent (v/v) and 2.0 mL of 7.5%  $\text{Na}_2\text{CO}_3$ . The reaction mixture was subsequently incubated at 45 °C for 40 min, and the absorbance measured at 760 nm by spectrophotometer. All tests were performed in triplicate. Gallic acid was used as a standard phenolic compound. The amount of total phenolic compounds in the extract was determined as mg of Gallic Acid Equivalent (GAE) per g dry weight (Sin-

gleton, Orthofer, & Lamuela-Raventos, 1999).

## 2.5 Determination of total flavonoid

A known volume (0.5 mL) of each extract ( $1\text{-}5\text{ mg mL}^{-1}$ ) was added to a 10 mL volumetric flask. Distilled water was added to make a volume of 5 mL. At zero time, 0.3 ml of 5 % w/v  $\text{NaNO}_2$  was added to the flask. After 5 min, 0.6 mL of 10 % w/v  $\text{AlCl}_3$  was added and after 6 min, 2 mL of 1M NaOH was added to the mixture followed by the addition of 2.1 mL distilled water. Absorbance was read at 510 nm against the blank (water) and flavonoid content expressed as mg Rutin equivalent/g. The test was carried out in triplicate (Zhishen, Mengcheng, & Jianming, 1999).

## 2.6 Determination of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging ability

Extracts of  $1\text{-}5\text{ mg mL}^{-1}$  each were mixed with 1 mL, 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min before measuring the absorbance at 516 nm. This was carried out in triplicate (Zhishen et al., 1999). Percentage of inhibition =  $\frac{A_0 - A_1}{A_0} \times 100$ , where  $A_0$  is the absorbance of the Trolox and  $A_1$  is the absorbance of the sample (Ursini, Maiorino, Morazzoni, Roveri, & Pifferi, 1994). The percentage inhibition was plotted against the corresponding sample concentration ( $1\text{-}5\text{ mg mL}^{-1}$ ) and  $\text{IC}_{50}$  calculated from the graph equation.

## 2.7 Determination of hydroxyl (OH) radical scavenging activity

The OH scavenging activity was determined by the method of Halliwell, Gutteridge, and Aruoma (1987). Exactly  $1\text{-}5\text{ mg mL}^{-1}$  of each extract of the samples were mixed with 1 mL of reaction mixture (100  $\mu\text{M}$   $\text{FeCl}_3$ , 104  $\mu\text{M}$  Ethylene-

diaminetetraacetic acid, 1.5 M H<sub>2</sub>O<sub>2</sub>, 2.5 M deoxyribose and 100 μM ascorbic acid in 10 mM KH<sub>2</sub>PO<sub>4</sub>-KOH, pH 7.4) and incubated for 1h at 37 °C. Thereafter, 1 mL of 0.5 % thiobarbituric acid in 0.025 M NaOH and 1 mL of 2.8 % trichloroacetic acid was added to the mixture and heated for 30 min at 80 °C before reading the absorbance at 532 nm against an appropriate blank solution. All tests were performed in triplicate. Ascorbic acid was used as a positive control. Percent inhibition of OH was calculated by the following expression: Percentage of inhibition =  $\frac{A_0 - A_1}{A_0} \times 100$ , where A<sub>0</sub> is the absorbance of the Ascorbic acid and A<sub>1</sub> is the absorbance of the sample. The percentage inhibition was plotted against corresponding sample concentration (1-5 mg mL<sup>-1</sup>) and IC<sub>50</sub> calculated from the graph equation.

## 2.8 Determination of nitric oxide (NO) scavenging activity

Briefly, 5 mM sodium nitroprusside in phosphate-saline was mixed with different concentrations of the extracts: 1-5mg mL<sup>-1</sup>, before incubation at 25 °C for 150 min. Thereafter, the reaction mixture was added to Greiss reagent (1 % sulfanilamide, 2 % H<sub>3</sub>PO<sub>4</sub> and 0.1 % naphthylethylenediamine dihydrochloride), before measuring the absorbance at 546 nm (Jagetia, Rao, Baliga, & Babu, 2004). Ascorbic acid was used as the control. All tests were performed in triplicate. The nitric oxide radicals scavenging activity of the fractions was calculated according to the following equation: Percentage of inhibition =  $\frac{A_0 - A_1}{A_0} \times 100$ , where A<sub>0</sub> is the absorbance of Ascorbic acid and A<sub>1</sub> is the absorbance in the presence of the fractions and ascorbic acid. The percentage inhibition was plotted against the corresponding sample concentration (1-5 mg mL<sup>-1</sup>) and IC<sub>50</sub> calculated from the graph equation.

## 2.9 Determination of nitric oxide (NO) scavenging activity

Values are presented as the mean ± SD of three replicates. ANOVA and LSD and Pearson cor-

relation analyses were performed using the commercial software SPSS 16.0

## 3 Results and Discussion

### 3.1 Results

#### Phenolic compounds

The HPLC profile of alcoholic extracts of *Launea taraxacifolia* revealed the presence of phenolic acids (gallic, caffeic, ellagic, and chlorogenic acids) and flavonoids (rutin, isoquercitrin, quercetin and kaempferol, coumarin, quercitrin and catechin) (Table 1, Figures 1 - 2).

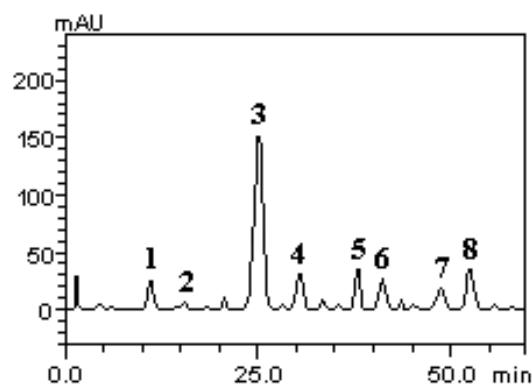


Figure 1: HPLC profile of a methanolic extract of *Launea taraxacifolia*. Gallic acid (peak 1), catechin (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), rutin (peak 5), isoquercitrin (peak 6), quercetin (peak 7) and kaempferol (peak 8)

#### Total phenol and total flavonoid contents

TPC and TFC of *L. taraxacifolia* for all extracts were in the range 13.54-133.20 and 1.21- 78.79 mg/100 g respectively, while TPC and TFC of *C. ruben* for all extracts were 9.45-81.64 and 0.85-60.61 mg/100g respectively. The methanolic extracts of both vegetables contained the highest concentrations of TPC but the aqueous extracts gave the highest values for TFC. There was a positive and strong correlation between the antioxi-

Table 1: Phenolic compounds in the alcoholic extracts of the vegetables

Compounds (mg/g)	Methanolic extracts		Ethanollic Extracts	
	<i>L. taraxacifolia</i>	<i>C. ruben</i>	<i>L. taraxacifolia</i>	<i>C. ruben</i>
Gallic Acid	10.95±0.03	4.30±0.02	0.37 ± 0.01	0.45 ± 0.01
Chlorogenic Acid	0±0.00	50.91±0.01	0.98 ± 0.03	0.91 ± 0.01
Caffeic Acid	72.30±0.01	26.81±0.02	4.35 ± 0.03	4.23 ± 0.03
Caffeic Acid <i>Derivative</i>	0±0.00	12.76±0.01	0±0.00	0±0.00
Coumarin	0±0.00	0±0.00	5.91 ± 0.01	4.37 ± 0.02
Ellagic Acid	18.67±0.01	0±0.00	0±0.00	0±0.00
Catechin	2.41±0.02	0±0.00	0±0.00	0±0.00
Rutin	19.53±0.03	9.07±0.01	3.47 ± 0.02	3.36 ± 0.01
Isoquercitrin	18.24±0.02	27.15±0.03	0±0.00	0±0.00
Quercitrin	0±0.00	12.83±0.01	3.69 ± 0.01	3.81 ± 0.01
Quercetin	12.07±0.03	39.61±0.01	6.03 ± 0.01	3.40 ± 0.02
Kaempferol	20.51±0.01	8.42±0.02	3.65 ± 0.03	3.95 ± 0.01

Results are expressed as mean ± standard deviations (SD) of three determinations

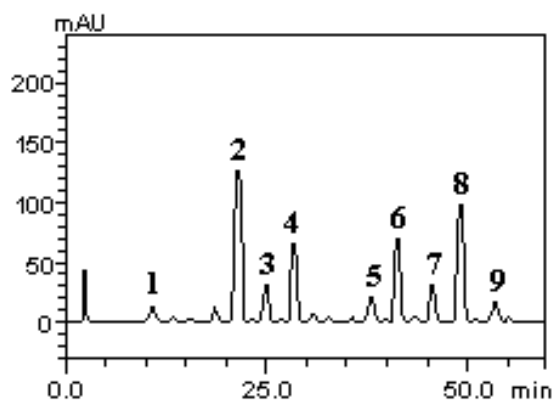


Figure 2: HPLC profile of a methanolic extract of *Crassocephalum rubens*. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), caffeic acid derivative (peak 4), rutin (peak 5), isoquercitrin (peak 6), quercitrin (peak 7), quercetin (peak 8) and kaempferol (peak 9)

dant activities and the TFC and TPC (Tables 2, 3 and 4).

### DPPH Radical Scavenging Property

The percentage inhibitions of DPPH in aqueous extracts were lower than in alcoholic extracts. All extracts demonstrated relatively appreciable concentration dependent increase, compared with Trolox (Tables 5 - 6). IC<sub>50</sub> values for free radicals are presented in Table 7.

### Hydroxyl Radical Scavenging Property

Aqueous extracts exerted a stronger scavenging capacity on OH in a dosage dependent manner than the alcoholic extracts and Ascorbic acid (Tables 5 - 6). The extracts showed significant *in vitro* antioxidant capacities.

### Nitric Oxide Radical Scavenging Property

The percentage inhibition of NO was higher in methanolic extracts for both *L. taraxacifolia* and *C. rubens* than in aqueous extracts except at 1 mg mL<sup>-1</sup> concentrations. Aqueous extracts however exhibited stronger scavenging activity than

Table 2: TFC and TPC of aqueous extracts (mg/100g)

	Conc. (mg/ml)	<i>L. taraxacifolia</i> Aq. extracts	<i>C. rubens</i> Aq. extracts	Reference standard (Rutin/Gallic Acid)
TFC	1	12.12 <sup>b</sup> ±0.01	6.06 <sup>a</sup> ± 0.02	34.85 <sup>c</sup> ±0.03
	2	21.21 <sup>a</sup> ±0.01	21.21 <sup>a</sup> ±0.01	63.64 <sup>b</sup> ±0.05
	3	48.49 <sup>b</sup> ±0.03	42.42 <sup>a</sup> ±0.05	104.55 <sup>c</sup> ±0.14
	4	63.64 <sup>b</sup> ±0.02	51.52 <sup>a</sup> ±0.01	136.36 <sup>c</sup> ±0.17
	5	78.79 <sup>b</sup> ±0.01	60.61 <sup>a</sup> ±0.03	181.82 <sup>c</sup> ±0.06
TPC	1	24.71 <sup>b</sup> ±0.03	14.83 <sup>a</sup> ±0.01	275.97 <sup>c</sup> ±0.09
	2	30.62 <sup>b</sup> ±0.02	18.78 <sup>a</sup> ±0.01	684.17 <sup>c</sup> ±0.11
	3	34.38 <sup>b</sup> ±0.2	30.71 <sup>a</sup> ±0.03	1038.66 <sup>c</sup> ±0.08
	4	47.27 <sup>b</sup> ±0.01	41.55 <sup>a</sup> ±0.02	1432.79 <sup>c</sup> ±0.15
	5	52.64 <sup>b</sup> ±0.03	50.08 <sup>a</sup> ± 0.01	1655.06 <sup>c</sup> ±0.12

Values represent means of triplicate readings ± SD. (n=3). Values with the same superscript along the row are not significantly different (n=3).

Table 3: TFC and TPC of alcohol extracts (mg/100g)

	Conc (mg/ml)	<i>L.taraxacifolia</i> (Methanolic Extract)	<i>C. rubens</i> (Methanolic Extract)	<i>L.taraxacifolia</i> (Ethanolic Extract)	<i>C. ruben</i> (Ethanolic Extract)	Reference standard (Rutin/Gallic Acid)
TFC	1	1.21 <sup>c</sup> ±0.02	0.93 <sup>b</sup> ±0.01	1.76 <sup>d</sup> ±0.02	0.85 <sup>a</sup> ±0.01	34.85 <sup>e</sup> ±03
	2	5.4 <sup>d</sup> ±0.01	1.25 <sup>a</sup> ±0.01	2.67 <sup>c</sup> ±0.01	1.58 <sup>b</sup> ±0.01	63.64 <sup>e</sup> ±0.05
	3	7.27 <sup>d</sup> ±0.01	1.84 <sup>a</sup> ±0.01	4.61 <sup>c</sup> ±0.02	2.24 <sup>b</sup> ±0.02	104.55 <sup>e</sup> ±0.14
	4	10.91 <sup>d</sup> ±0.04	4.85 <sup>b</sup> ±0.01	5.88 <sup>c</sup> ±0.02	3.15 <sup>a</sup> ±0.03	136.36 <sup>e</sup> ±0.17
	5	12.73 <sup>d</sup> ±0.04	8.49 <sup>c</sup> ±0.01	7.70 <sup>b</sup> ±0.03	4.30 <sup>a</sup> ±0.03	181.82 <sup>e</sup> ±0.06
TPC	1	38.67 <sup>d</sup> ±0.02	21.48 <sup>c</sup> ±0.01	13.54 <sup>b</sup> ±0.03	9.45 <sup>a</sup> ±0.01	275.97 <sup>e</sup> ±0.09
	2	51.56 <sup>d</sup> ±0.01	34.38 <sup>c</sup> ±0.02	24.06 <sup>b</sup> ±0.01	18.91 <sup>a</sup> ±0.02	684.17 <sup>e</sup> ±0.11
	3	88.09 <sup>d</sup> ±0.02	55.86 <sup>c</sup> ±0.02	34.38 <sup>b</sup> ±0.01	29.43 <sup>a</sup> ±0.02	1038.66 <sup>e</sup> ±0.08
	4	98.49 <sup>d</sup> ±0.01	68.75 <sup>c</sup> ±0.01	48.12 <sup>b</sup> ±0.04	36.95 <sup>a</sup> ±0.04	1432.79 <sup>e</sup> ±0.15
	5	133.20 <sup>d</sup> ±0.16	81.64 <sup>c</sup> ±0.03	59.73 <sup>b</sup> ±0.02	48.55 <sup>a</sup> ±0.03	1655.06 <sup>e</sup> ±0.12

Values represent means of triplicate readings ± SD. (n=3).

Table 4: Pearson's correlation coefficient between TFC, TPC and antioxidant assays

		Samples	Pearson correlation		
		r value	DPPH	OH	NO
Aqueous	TFC	<i>L. taraxacifolia</i>	.965**	.989**	.995**
	TPC		.995**	.991**	.945*
	TFC	<i>C. ruben</i>	.972**	.907*	1.000**
	TPC		.975**	.962**	.976**
Methanolic	TFC	<i>L. taraxacifolia</i>	.963**	.985**	.926*
	TPC		.891*	.910*	.825
	TFC	<i>C. ruben</i>	.966**	.846	.432
	TPC		.994**	.895*	.473
Ethanolic	TFC	<i>L. taraxacifolia</i>	.997**	.958*	.996**
	TPC		.999**	.952*	.994**
	TFC	<i>C. ruben</i>	.990**	.978**	.978**
	TPC		.978**	.957*	.994**
Control			.965**	.968**	.987**

\*\*Correlation is significant at 0.01 level (2-tailed), \* Correlation is significant at 0.05 level (2-tailed).

Table 5: Antioxidant properties of the aqueous extracts (%)

Samples concentrations (mg/ml)		<i>L. taraxacifolia</i> Aq. Extracts	<i>C. rubens</i> Aq. Extracts	Reference standard Trolox/Ascorbic acid
1	DPPHRSP	14.48 <sup>b</sup> ± 0.04	13.66 <sup>a</sup> ± 0.13	53.65 <sup>c</sup> ± 0.05
2		19.23 <sup>a</sup> ± 0.01	20.77 <sup>b</sup> ± 0.02	63.49 <sup>c</sup> ± 0.05
3		23.10 <sup>a</sup> ± 0.05	25.87 <sup>b</sup> ± 0.07	77.86 <sup>c</sup> ± 0.01
4		34.64 <sup>a</sup> ± 0.05	36.65 <sup>b</sup> ± 0.09	81.58 <sup>c</sup> ± 0.05
5		42.86 <sup>b</sup> ± 0.03	37.85 <sup>a</sup> ± 0.05	92.38 <sup>c</sup> ± 0.03
1	OHRSP	40.83 <sup>a</sup> ± 0.10	52.34 <sup>c</sup> ± 0.03	47.64 <sup>b</sup> ± 0.02
2		49.58 <sup>a</sup> ± 0.10	58.54 <sup>c</sup> ± 0.02	54.28 <sup>b</sup> ± 0.06
3		60.42 <sup>a</sup> ± 0.03	63.43 <sup>b</sup> ± 0.05	67.44 <sup>c</sup> ± 0.03
4		74.17 <sup>c</sup> ± 0.06	73.64 <sup>b</sup> ± 0.17	72.47 <sup>a</sup> ± 0.01
5		86.67 <sup>b</sup> ± 0.04	91.74 <sup>c</sup> ± 0.19	75.48 <sup>a</sup> ± 0.04
1	NORSP	38.47 <sup>a</sup> ± 0.11	39.44 <sup>b</sup> ± 0.11	52.68 <sup>c</sup> ± 0.11
2		45.85 <sup>a</sup> ± 0.19	51.28 <sup>b</sup> ± 0.03	55.88 <sup>c</sup> ± 0.02
3		69.18 <sup>b</sup> ± 0.01	68.57 <sup>a</sup> ± 0.17	69.77 <sup>c</sup> ± 0.01
4		77.66 <sup>c</sup> ± 0.02	76.33 <sup>b</sup> ± 0.04	72.87 <sup>a</sup> ± 0.01
5		86.15 <sup>c</sup> ± 0.05	83.90 <sup>b</sup> ± 0.19	75.76 <sup>a</sup> ± 0.02

Values represent means of triplicate readings ± SD. Values with the same (n=3).

Table 6: Antioxidant properties of the alcoholic extracts (%)

Conc (mg/ml)		<i>L. taraxacifolia</i>		<i>C. rubens</i>		Reference standard (Trolox/ Ascorbic Acid)
		Methanolic extracts	Ethanollic extracts	Methanolic extracts	Ethanollic extracts	
1	DPPHRSP	33.84 <sup>d</sup> ±0.06	22.81 <sup>b</sup> ±0.01	33.39 <sup>c</sup> ±0.07	14.53 <sup>a</sup> ±0.01	53.65 <sup>e</sup> ±0.05
2		4d.89 <sup>b</sup> ±0.03	28.16 <sup>b</sup> ±0.04	40.10 <sup>c</sup> ±0.04	20.62 <sup>a</sup> ±0.04	63.49 <sup>e</sup> ±0.05
3		45.08 <sup>c</sup> ±0.04	32.70 <sup>b</sup> ±0.03	47.76 <sup>d</sup> ±0.10	25.62 <sup>a</sup> ±0.01	77.86 <sup>e</sup> ±0.01
4		47.62 <sup>c</sup> ±0.01	36.24 <sup>a</sup> ±0.05	55.14 <sup>d</sup> ±0.01	39.65 <sup>b</sup> ±0.16	81.58 <sup>e</sup> ±0.05
5		48.41 <sup>c</sup> ±0.05	41.14 <sup>a</sup> ±0.02	62.68 <sup>d</sup> ±0.07	47.30 <sup>b</sup> ±0.03	92.38 <sup>e</sup> ±0.03
1	OHRSP	13.33 <sup>b</sup> ±0.2	11.67 <sup>a</sup> ±0.3	17.79 <sup>d</sup> ±0.06	14.42 <sup>c</sup> ±0.06	47.64 <sup>e</sup> ±0.02
2		21.67 <sup>d</sup> ±0.06	20.60 <sup>b</sup> ±0.02	21.54 <sup>c</sup> ±0.01	14.43 <sup>a</sup> ±0.18	54.28 <sup>e</sup> ±0.06
3		21.97 <sup>b</sup> ±0.03	28.33 <sup>d</sup> ±0.01	24.69 <sup>c</sup> ±0.19	19.54 <sup>a</sup> ±0.06	67.44 <sup>e</sup> ±0.03
4		26.58 <sup>c</sup> ±0.06	24.17 <sup>a</sup> ±0.02	28.13 <sup>d</sup> ±0.01	26.38 <sup>b</sup> ±0.05	72.47 <sup>e</sup> ±0.01
5		29.50 <sup>a</sup> ±0.14	30.83 <sup>b</sup> ±0.06	40.27 <sup>d</sup> ±0.05	34.94 <sup>c</sup> ±0.20	75.48 <sup>e</sup> ±0.04
1	NORSP	31.77 <sup>d</sup> ±0.31	28.24 <sup>c</sup> ±0.05	23.57 <sup>b</sup> ±0.03	21.99 <sup>a</sup> ±0.13	52.68 <sup>e</sup> ±0.11
2		61.18 <sup>e</sup> ±0.02	58.82 <sup>d</sup> ±0.04	27.06 <sup>a</sup> ±0.06	33.89 <sup>b</sup> ±0.13	55.88 <sup>c</sup> ±0.02
3		95.88 <sup>e</sup> ±0.14	87.06 <sup>d</sup> ±0.08	30.77 <sup>a</sup> ±0.14	39.39 <sup>b</sup> ±0.02	69.77 <sup>c</sup> ±0.01
4		116.47 <sup>e</sup> ±0.09	114.12 <sup>d</sup> ±0.01	33.52 <sup>a</sup> ±0.08	43.44 <sup>b</sup> ±0.04	72.87 <sup>c</sup> ±0.01
5		151.76 <sup>e</sup> ±0.08	143.53 <sup>d</sup> ±0.05	37.61 <sup>a</sup> ±0.01	49.93 <sup>b</sup> ±0.04	75.76 <sup>c</sup> ±0.02

Values represent means of triplicate readings ± SD. (n=3).

Table 7: IC<sub>50</sub> values for free radicals in mg ml<sup>-1</sup>

Solvents	Samples	DPPH. IC <sub>50</sub>	OH. IC <sub>50</sub>	NO. IC <sub>50</sub>
Aqueous	<i>L. taraxacifolia</i>	6.59	1.94	12.8
	<i>C. ruben</i>	6.21	1.09	9.89
Methanolic	<i>L. taraxacifolia</i>	4.91	10.4	1.59
	<i>C. ruben</i>	6.98	9.42	1.73
Ethanollic	<i>L. taraxacifolia</i>	3.29	2.46	4.88
	<i>C. ruben</i>	5.42	2.91	8.65
Control		0.51	1.18	0.56
		TROLOX	VIT C	VIT C



the ethanolic extracts. The antioxidant activities of all were appreciable, when compared with Ascorbic acid, a reference standard (Tables 5 - 6).

### 3.2 Discussion

HPLC fingerprinting of methanolic and ethanolic extracts of *L. taraxacifolia* and *C. rubens* revealed the presence of phenolic acids; gallic acid and caffeic acid. Chlorogenic Acid which was the most abundant phenolic acid in the methanolic extract of *C. rubens* was absent only in the methanolic extract of *L. taraxacifolia* but detected in small concentrations in the ethanol extracts of both vegetables. Ellagic acid was, however, detected only in the methanolic extract of *L. taraxacifolia* while a caffeic acid derivative was also found only in the methanolic extracts of *C. rubens*. Rutin, quercetin and kaempferol are flavonoid compounds detected in methanolic and ethanolic extracts of both vegetables (Table 1). Phenolic compounds and flavonoids make up a unique category of plant phytochemicals especially in terms of their wide ranging potential health-beneficial properties. In particular, phenolic compounds have been reported to modulate intracellular signaling, the activity of target enzymes and gene expression (Kumari & Jain, 2012). Flavonoids have antioxidant activity and a range of health promoting effects such as anti-allergic, anti-inflammatory, anti-thrombotic, vasoprotective, tumour inhibitory and anti-viral. These effects have been associated with the influence of flavonoids on the arachidonic acid metabolism (Ajiboye, Ibukun, Edobor, Ojo, & Onikanni, 2013; Trease & Evans, 2002). Isoquercitrin was detected in the methanolic but not in the ethanolic extracts of both *L. taraxacifolia* and *C. rubens*; whilst coumarin was found in ethanolic but not the methanolic extracts. Methanolic extracts of *L. taraxacifolia* lacked the presence of quercitrin but contained catechins which are powerful antioxidants that have been reported to cause reduction in glycemia (blood glucose levels) either by inhibiting intestinal glucose absorption (Shimizu, Kobayashi, Suzuki, Satsu, & Miyamoto, 2000), inducing  $\beta$  cell regeneration (Kim et al., 2003) or enhancing insulin

activity (Anderson & Polansky, 2002). Based on the findings here and in previous studies, *L. taraxacifolia* and *C. rubens* extracts may be of significant value in the management of diabetes mellitus.

TFC and TPC results revealed concentration dependent increase (Tables 2 and 3), and *L. taraxacifolia* for all extracts gave significantly ( $p < 0.05$ ) higher values of TPC (13.54-133.20) than *C. ruben* (9.45-81.64). The methanolic extracts of both vegetables contained the highest concentrations of TPC. This was similar to the observation of Corpuz, Osi, and Santiago (2013) where aqueous extracts of *S. siliquosum* presented the lower amount of TPC in  $\text{mg g}^{-1}$  than alcoholic extracts. TFC of *L. taraxacifolia* for all extracts were also significantly ( $p < 0.05$ ) higher (1.21- 78.79  $\text{mg}/100\text{g}$ ) than *C. ruben* (0.85- 60.61  $\text{mg}/100\text{g}$ ). The aqueous extracts of both however were significantly ( $P < 0.05$ ) higher than the alcoholic extracts in this study. The vegetables contained appreciable TPC and TFC though in significantly lower concentrations than gallic acid and rutin which were used as the standards in  $\text{mg GAE}/100\text{g}$  and  $\text{RE}/100\text{g}$  dry matter. 28.38 ( $\text{mg GAE}/\text{g}$  dry matter) and 0.97 ( $\text{mg CTE}/\text{g}$  dry matter) reported for TPC and TFC of methanolic extracts of *L. taraxacifolia* by Adetutu, Olorunnisola, and Oyewo (2013) were higher than 13.54-133.20 ( $\text{mg GAE}/100\text{g}$ ) and 12.12- 78.79 ( $\text{mg RE}/100\text{g}$ ) respectively obtained for the same vegetable in this study.

DPPH analysis demonstrates the ability of the vegetables under study to act as donors of hydrogen atoms as presented in Tables 5-6. Percentage inhibitions by the aqueous extracts were significantly ( $p < 0.05$ ) lower than the alcoholic extracts, where the ethanolic extracts of both vegetables demonstrated the strongest scavenging power and *L. taraxacifolia* for all extracts exerted a significantly ( $p < 0.05$ ) stronger scavenging power than *C. Ruben*. These are in agreement with the trend observed for TPC and supported by a strong and positive correlation between both TFC and TPC and DPPH at a 0.01- 0.05 level of significance (Table 4). 1 and 5  $\text{mg mL}^{-1}$  methanolic extracts of *C. rubens* gave 0 and 37.6 % DPPH scavenging activities in an earlier study by Iwalewa et al. (2005) which falls within the range of 22.8 – 41.1 % obtained for the same ex-

tract in this study. The Trolox standard exhibited significantly ( $p < 0.05$ ) stronger inhibition than the extracts of vegetable. The  $IC_{50}$  value, which is inversely proportional to the antiradical activity, is the concentration of sample in  $mg\ mL^{-1}$  required for 50 % scavenging of radicals. The  $IC_{50}$  values ranged from 3.30 in ethanolic extract of *L. taraxacifolia* to 6.98  $mg\ L^{-1}$  in the methanolic extract of *C. rubens*. The extract using ethyl acetate of *Crassocephalum bauchiense* was reported to exhibit strong scavenging activity on DPPH, with  $IC_{50}$  of 28.57 - 389.38  $\mu g\ mL^{-1}$  for different fractions by Mouokeu, Ngono Ngane, Njateng, Kamtchueng, and Kuate (2014).

The OH scavenging activity was measured by the ability of the extracts to scavenge the hydroxyl radicals generated by the  $Fe^{3+}$ -ascorbate-EDTA- $H_2O_2$  system in the Fenton reaction (Ilavarasan, Mallika, & Venkataraman, 2006). The aqueous extracts exerted significantly ( $p < 0.05$ ) stronger scavenging capacity on hydroxyl radicals in a dosage dependent manner than the alcoholic extracts and ascorbic acid. High TFC of the aqueous extracts may be responsible since phenolic compounds of plants belong to different classes of phenols and vary in their antioxidant strengths. The results of this study clearly indicate a positively strong correlation between both TFC and TPC of the extracts and the anti-hydroxyl radical capacities of the extracts as shown in Table 4. The extracts contained a considerable amount of phenolics and possess significant *in vitro* antioxidant capacities (Tables 5 -6). The aqueous extract of *C. ruben* demonstrated the strongest inhibitory power against OH radical with an  $IC_{50}$  value of 1.09  $mg\ ml^{-1}$ , which was stronger than ascorbic acid used as a reference standard.

Although, nitric oxide plays a vital role in various inflammatory processes, high levels of these radicals are toxic to tissue and contribute to the vascular collapse, various carcinoma and ulcerative colitis (Rajan et al., 2011). The results of this study showed that the percentage inhibition was significantly ( $p < 0.05$ ) higher in methanolic extracts of both *L. taraxacifolia* and *C. rubens* than aqueous extracts except at 1  $mg/mL$  concentrations. However, aqueous extracts exhibited stronger scavenging activity than the ethanolic extracts, which is also in line with the trend ob-

served for TPC. All extracts of the vegetables compared favorably with vitamin C in their inhibitory effect on nitric oxide. The methanolic extract of *L. taraxacifolia* with an  $IC_{50}$  value of 1.60 exhibited the strongest anti-radical ability against nitric oxide in this study.

The ability of *L. taraxacifolia* and *C. rubens* to effectively inhibit free radicals (OH, NO, DPPH) and their reducing properties in this study may be attributed to the presence of phenolic compounds with potent antiradical properties. These vegetables possess high antioxidant potential to boost the body antioxidants and protect humans against detrimental oxidative effects caused by free radicals (Shukla, Mehta, Bajpai, & Shukla, 2009; Corpuz et al., 2013).

## 4 Conclusions

The results of this study clearly indicate that aqueous and alcoholic extracts of *L. taraxacifolia* and *C. rubens* possess significant *in vitro* antioxidant capacities. Generally, strong and positive associations were observed between antioxidant activities and the total phenolic content as well as the total flavonoids' content of the vegetable extracts, although the order of antioxidant potency of each extract evaluated by different models does not follow the same pattern. These observations were in agreement with several studies which associated phenolic compounds with antioxidant activity. The reported potential health benefits of *L. taraxacifolia* and *C. rubens* may be attributed to the considerable amount of various phenolic acids and flavonoids detected in them. *L. taraxacifolia* and *C. rubens* can therefore be viewed as potential health foods, and their cultivation and utilization should be encouraged.

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