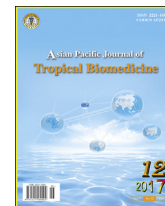




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journal homepage: www.elsevier.com/locate/apjtbOriginal article <https://doi.org/10.1016/j.apjtb.2017.10.008>Phytochemical and antioxidant activities of *Rumex crispus* L. in treatment of gastrointestinal helminths in Eastern Cape Province, South AfricaOladayo Amed Idris, Olubunmi Abosede Wintola^{*}, Anthony Jide Afolayan

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ABSTRACT

Objective: To evaluate the antioxidant activities and phytochemical content of the leaf and root extracts of *Rumex crispus* using the solvents extraction; methanol extract, ethanol extract, acetone extract (ACE), and water extract.

Methods: Total flavonoids content, total phenolic content, and total proanthocyanidin were evaluated using spectrophotometric equivalents of the standards, quercetin, gallic acid and catechin respectively. The antioxidant activities of the plant extracts were determined using ABTS, DPPH, ferric reducing antioxidant power, total antioxidant capacity and nitric oxide scavenging assays.

Results: The flavonoids and phenols contents of the extracts were in the range of (19.39 ± 4.08) to (526.23 ± 17.52) mg QE/g and (16.95 ± 12.03) to (240.68 ± 3.50) mg GAE/g, respectively. ACE of the leaf has the highest value of total flavonoids content (526.23 ± 17.52) mg QE/g while ACE of the root has the highest value of total phenolic content (240.68 ± 3.50) mg GAE/g. The highest content of total proanthocyanidin (645.38 ± 1.33) mg CE/g was in ACE of the root. Significant amounts of saponin and alkaloid were also present in the root and leaf extracts. All solvent fractions showed significant antioxidant activities ($P < 0.05$) with ACE of the root having the highest scavenging value as shown in DPPH, ABTS, total antioxidant capacity, nitric oxide and ferric reducing antioxidant power ($IC_{50} = 0.014$ mg/mL, <0.005 mg/mL, 0.048 mg/mL, 0.067 mg/mL, and 0.075 mg/mL, respectively).

Conclusions: In this study, the mean phytochemical content of the root of *Rumex crispus* is higher than that of the leaf and this may have contributed to its high antioxidant activities. This may also justify the frequent use of the root more than the leaves in traditional medicine for the cure of helminthic infections.

1. Introduction

Plants have been used for the therapy of many diseases since ancient time. Plant's roots, seeds, bark, leaves, or flowers could be used for remedial purposes. In present civilized world, synthetic medicines are readily available and they are efficient in the treatment of various diseases but some people still choose herbal medicines above the synthetic drugs because they are less harmful [1]. Kumarasingha *et al.* [2], reported that natural compounds from

plants provide a prospect in the search for new drugs which are effective, safe and with better pharmacological action than the synthetic drugs. Several compounds, found and isolated from plants have shown properties such as anticancer, anthelmintic, analgesic, antibacterial, anti-inflammatory, antiviral and many other biological activities to a lesser or greater extent [3,4]. Research in the area of ethnobotany and medicinal plants as used by folklore medicine shows that plants are better and safer source of drug for certain diseases and pests [5]. There are several isolated phytochemical compounds which include phenols and phenolic glycosides, flavonoids, saponins and cyanogenic glycosides, tannins, nitrogen compounds (amines, betalains, and alkaloids), terpenoids, stilbenes and some other endogenous metabolites [6].

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There has been confirmation that food rich in natural antioxidants due to its phytochemical constituents is linked with reducing risks of some diseases, mostly cardiovascular and cancer [7]. Damage of biological molecules can be significantly reduced by antioxidants by decreasing oxidative stress [6]. Reactive oxygen species are compounds formed from oxygen metabolism during oxidative stress. These highly reactive and free molecules produced during oxygen metabolisms such as organic peroxide (RO[•]), hydroxyl radical (•OH), and superoxide radicals (O₂^{•-}) can cause severe destruction to cells and tissues. Propyl gallate, butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole are among synthetic antioxidants but these compounds have been reported to cause external and internal bleeding in guinea pigs and rats at high concentration [8,9].

There is, thus a need for antioxidant with a different mechanism of action. This has led to the usage of antioxidants derived from plant's bioactive phytochemicals such as flavonoids which are proven to be efficient in scavenge of free radicals [6]. The bioactive constituents of medicinal plants can be extracted with different methods and then subjected to evaluation. There are reports of significant differences in physiological activities of plant extracts which depends upon the extraction methods and it emphasizes the importance of choosing a fitting extraction method for a specific purpose [7].

The study plant belongs to the genus '*Rumex*' which refers to acid; and the word 'crispus' means curled, which alludes to the wavy and curly leaves of this plant species and gave it the common name 'curled dock'. *Rumex crispus* (*R. crispus*) L. belongs to the family Polygonaceae and it is an herb which grows between 40 cm and 120 cm tall. It is a perennial plant, which can survive for several years by means of a fleshy taproot. The root could be up to 4 cm in width, reaching a depth of 150 cm or more in the soil. The leaves are hairless, it has long inflorescence or flower stalk that bears seeds in a cluster. It grows mostly in a wet ground as a weed. The infusion or decoction of *R. crispus* is commonly used in folk medicines by natives of South Africa for the treatment of helminths, wound, internal bleeding and vascular diseases especially in the rural area of Eastern Cape Province [10]. The objective of this study is to carry out quantitative phytochemical screening and antioxidant evaluation of *R. crispus*.

2. Materials and methods

2.1. Plant selection

The ethnobotanical survey of South Africa's Eastern Cape Province was done by Wintola and Afolayan [10]. Ethnobotanical data was collected in Amathole Municipal of Ngqushwa, Amahlathi, Buffalo Nxuba, Nkonkobe, Greet Kie, Mbashe and Mnguma. The Province's geographical location is within longitudes 22°45' to 30°15' E and latitudes 30°00'N to 34°15'S [11]. *R. crispus* was among the foremost cited anthelmintic plants surveyed by Wintola and Afolayan [10] which was selected for this study.

2.2. Plant collection and authentication

Plant specimen was obtained from the natural habitat in Alice, Eastern Cape of South Africa and was authenticated by a

renowned taxonomist. A sample of the specimen (Idr-Med-2017/03) was placed at the herbarium (Giffen) of the University of Fort Hare, for future citation.

2.3. Extract preparation

The aerial part and the root of the plant were dried separately in an oven at 40 °C continuously until a permanent weight was reached. The dried plant material was pulverized to powder with an industrial electric blender (Polymix PX-MFC90D Switzerland) and stored in the refrigerator at a temperature of 4 °C until use. Extraction was done on the fine-grounded plant material using the following solvents: water extract (WAE), ethanol extract (ETE), acetone extract (ACE), and methanol extract (MEE). All extractions were prepared by macerate 60 g of plant material in 1 000 mL of the solvents and shake for 48 h with a mechanical shaker (Gallenkamp Orbital Shaker). The mixture was filtered using a Buchner funnel, vacuum pump, and Whatman No. 1 filter paper. Thereafter, the collected filtrate of WAE was chilled at -40 °C with refrigerant (PolyScience AD15R-40-A12E, USA) and freeze-dried with a dryer (Savant vapour trap, RV-T41404, USA) for 48 h. The filtrate of ETE, ACE and MEE were concentrated with a rotary evaporator (Strike-202 Steroglass, Italy) at the boiling point of each solvent.

Percentage yield of MEE, ETE, ACE and WAE in leaf of *R. crispus* was determined and recorded as follows: 11.50% ± 3.72%, 4.21% ± 1.05%, 2.43% ± 1.16%, 15.37% ± 2.98%, respectively; in root was 17.08% ± 2.73%, 8.08% ± 3.02%, 3.48% ± 0.15% and 14.97% ± 2.94%, respectively. Thereafter dried extracts were stored in universal bottles and kept at 4 °C.

2.4. Chemicals and reagents

All chemicals and reagents used during the study were of standard grade. Quercetin dehydrate, rutin, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), sodium acetate, 1,1-diphenyl-2-picryl-hydrazyl, potassium persulfate, sodium nitroprusside, ascorbic acid, gallic acid, BHT, ferric chloride, NaOH, ferrous chloride hexahydrate, Na₂CO₃, AlCl₃, potassium acetate, Folin-Ciocalteu reagent, potassium iodide, acetone, ethanol, methanol, (H₂SO₄, ammonium solution, HCl, glacial acetic acid, NaCl, K₃Fe (CN)₆, diethyl ether, buttan-1-ol, trichloroacetic acid, sulphanilamide, and 1-naphthylethylenediamine.

2.5. Qualitative phytochemical screening

2.5.1. Total phenolic content

In each extract, the total phenol content was evaluated using folin ciocalteu reagent and procedure was adopted as described [12] with slight modification. The stock of extracts and gallic acid standard were prepared in ratio 1:1 mg/mL in methanol. An aliquot of the stock (extract) was added in separate tubes to 2.5 mL of folin ciocalteu reagent, and the mixture was diluted with distilled water in ratio 1:10 v/v and 2 mL of 7.5% w/v anhydrous NaCO₃ was added after. The mixture was mixed with a vortexer for 60 s and allowed to incubate in water bath for 30 min at 40 °C. Total phenol content was evaluated by taking readings of the mixture's absorbance at 765 nm using Hewlett Packard VR-2000 spectrophotometer. The results was taken in triplicate and were estimated in

milligram per gram of extract's total phenol content in gallic acid equivalent (mg GAE/g) using the standard curve: $y = 9.0139x$, $R^2 = 0.9919$. Where R is the determined coefficient, x is the concentration, and y is the absorbance.

2.5.2. Total flavonoids content

The total flavonoids content of the sample were measurable as a result of the formation of a complex flavonoid-aluminum using the $AlCl_3$ assay, as described by Oyedemi *et al.* [13]. A volume of 2 mL distilled water was added to 0.15 mL of 5% $NaNO_3$ and an aliquot of the extract was added. A latent period of 5 min was allowed and 0.15 mL of 10% $AlCl_3$ was added, followed by 1 mL of 4% $NaOH$ after a quiescent period of 5 min. The mixture was vortexed and put in an incubator for 15 min at 40 °C. A standard solution of quercetin was prepared with varying concentrations using the same procedure. The absorbance of the mixture was measured at 510 nm and total flavonoids content was expressed as mg/g quercetin equivalents (mg QE/g) of extract: $y = 1.2315x$, $R^2 = 0.9984$.

2.5.3. Total flavonols content

Total flavonols was appraised using aluminum chloride assay as described by Kumaran and Karunakaran [14]. A volume of 2 mL each of the plant extract and aqueous $AlCl_3$ solution (20% in ethanol) was dissolved in 3.0 mL of sodium acetate (5% in distilled water). The solution of the mixture was vortex and incubated at 20 °C for 2.5 h. The absorbance of triplicate mixtures was read at 440 nm and results were expressed as equivalents of quercetin in mg QE/g dry weight using the equation from the standard curve: $y = 18.367x$, $R^2 = 0.9987$.

2.5.4. Total proanthocyanidin

The value of proanthocyanidin was estimated based on the procedure described by Unuofin *et al.* [15], with a minor modification. The experiment was done in triplicate. An aliquot volume of 0.5 mL of the test sample was mixed with 3 mL of vanillin-methanol (4% w/v), followed by 1.5 mL of HCl . The solution was vortex and allows to incubate for 15 min at 27 °C. The spectrophotometric absorbance was read at 500 nm. Total proanthocyanidin was expressed as mg/g dry weight of catechin equivalent (mg CE/g) of the extract; $y = 0.0013x$, $R^2 = 0.9836$.

2.5.5. Saponin

Saponin content of the plant sample was evaluated with the procedure previously described [16] with modification. One gram of the pulverized sample was macerated in 40 mL of 20% ethanol. The mixture was continuously stirred and incubated for 4 h at 55 °C in a water bath. The mixture was filtered with funnel, vacuum pump, and filter paper. Residue was collected and re-extracted with 20 mL of 20% ethanol. The volume of filtrate collected was thereafter reduced at 90 °C in a water bath and the concentrate was transferred into a 200 mL separating funnel. A volume of 20 mL of diethyl ether was added and mixed vigorously. The lower fraction was collected while ether layer (upper) was discarded. A volume of 20 mL of butan-1-ol was added, mixed vigorously, followed by 5 mL of 5% aqueous $NaCl$. The butan-1-ol fraction (upper) was collected and

evaporated to constant weight in the oven. The saponin content in the sample was evaluated by the equation:

$$\text{Amount of saponin} = \frac{\text{Weight of fraction}}{\text{Weight of pulverize sample}} \times 100$$

2.5.6. Alkaloids determination

The alkaloid content was determined as the procedure was previously described [17]. Two grams of the pulverized plant specimen was weighed in a bottle containing 100 mL of 20% acetic acid in ethanol and macerated for 4 h. The mixture was filtered and the filtrate was concentrated in a water bath at 55 °C. Few drops of NH_4OH solution were added into the concentrated extract till the precipitate was complete. The precipitate was allowed to settle down and filtered through a pre-weigh filter paper. The residue collected on filter paper was weighed and the alkaloid content was calculated with the equation:

$$\text{Amount of alkaloid} = \frac{\text{Weight of precipitate}}{\text{Weight of pulverize sample}} \times 100$$

2.6. Evaluation of antioxidant activity

The antioxidant capacity of the root and leaf of *R. crispus* were determined by the standard methods; DPPH free radical scavenging, ABTS, total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP) assays and nitric oxide (NO). The experiment was done in triplicate.

2.6.1. DPPH radical scavenging assay

The capability of the plant sample to scavenge for free radical was measured with the stable radical of DPPH using method as described [6], with minor modification. A volume of 0.135 mM DPPH solution was prepared with methanol in a dark bottle and 2.5 mL of DPPH solution was mixed with different concentrations (equivalent to 0.005, 0.01, 0.02, 0.04, 0.08 mg/mL respectively) of 2.5 mL of extract and standards (Gallic acid, BHT and quercetin) dissolved in methanol. The mixtures were vortexed and kept in a dark room for 45 min at 27 °C. Inhibition of DPPH radical by the samples was measured at absorbance 517 nm against the blank and positive controls (Standard drugs). The activity of the radical scavenging ability of the tested samples was extrapolated in inhibitory percentage using the formula:

$$\% \text{ DPPH inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

The extract's half-inhibitory concentration (IC_{50}) was computed from the plot of scavenging effect in percentage against the equivalent extract concentration. In a linear regression of the plotted graph, the abscissa represented the concentration of test samples and the ordinate represents the mean percent of inhibitory ability from three replicates.

2.6.2. ABTS radical scavenging activity

The antioxidant capacity determined by ABTS radical ($ABTS^{+}$) was absorbed at 743 nm, a method described by Oyedemi *et al.* [18]. The ABTS radical was formed by the

mixture of 7 mM ABTS stable radical aqueous liquid with 2.45 mM K₂S₂O₈ in ratio 1:1 and kept away from light for 12–18 h at 27 °C. Prior to assay, ABTS solution was mixed with methanol (1:50 v/v) until an absorbance of 0.700 ± 0.005 was reached at 734 nm using a UV–visible spectrophotometer. Plant extracts and standard drug solutions of varying concentrations (0.005–0.08 mg/mL) were reacted with ABTS (1 mL) in test tubes in a dark room for 7 min and the absorbance was measured at 734 nm against the methanol (Blank). The percentage inhibition of both samples and standards were calculated.

$$\% \text{ ABTS scavenging} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

The concentration of the sample providing IC₅₀ was evaluated by plotting a graph of percentage inhibition of ABTS^{•+} by the sample against the corresponding sample concentration.

2.6.3. Phosphomolybdenum assay

The TAC of plant samples was measured due to the formation of sample-phosphomolybdenum complex as described [19,20]. An aliquot volume of 0.3 mL of plant sample extract was added to 3 mL of reagent (28 mM sodium phosphate, 0.6 M sulfuric acid and 4 mM ammonium molybdate) and different standard drugs [Rutin, gallic acid and butylated hydroxytoluene (BTH)] at varying range of concentration of 0.025–0.400 g/mL. The mixture was incubated in a water bath for 90 min at 95 °C. At 695 nm, the absorbance of the mixture was measured against the blank (Methanol) with a spectrophotometer. The percentage of inhibition of sample was calculated and the sample inhibitory concentration providing IC₅₀ was extrapolated by plotting percentage inhibition of sample against the corresponding sample concentration.

$$\% \text{ TAC inhibition} = \frac{\text{Sample absorbance} - \text{Control absorbance}}{\text{Sample absorbance}} \times 100$$

2.6.4. FRAP

The ferric reducing power of the plant extracts was appraised by the procedure described by Jayanthi and Lalitha [21]. A FRAP reagent was prepared by mixing 2.5 mL of K₃Fe (CN)₆ with 2.5 mL of 0.2 M phosphate buffer (Mixture of 62.5% monobasic and 37.5% dibasic: pH 6.6) in 1% (w/v). A varying concentration (0.025, 0.05, 0.1, 0.2 and 0.4 mg/mL) of the standard drugs (ruin, gallic acid, and BTH) and the plant extracts were added to the FRAP reagent. The mixture was allowed to incubate at 50 °C for 20 min. After incubation, a volume of 2.5 mL of 10% trichloroacetic acid was added to the mixture and the mixture was thereafter centrifuged for 10 min at 3 000 rpm. A freshly prepared 0.1% FeCl₃, distilled water and supernatant from the centrifuge were added in the ratio of 1:5:5 then incubated for 10 min at room temperature. Ferric reducing ability of the plant samples was measured at 700 nm against the methanol blank. The values of the results were expressed in mg Fe (II) equivalent/g extract dry mass.

$$\% \text{ FRAP} = \frac{\text{Sample absorbance} - \text{Control absorbance}}{\text{Sample absorbance}} \times 100$$

2.6.5. NO scavenging activity

The inhibitory capability of samples against NO radicals was evaluated using the method described by Ebrahimzadeh *et al.* [22], with little modification. A volume of 10 mM of phosphate buffer saline (pH 7.4) was dissolved in 10 mM sodium nitroprusside and 2 mL of the solution was added to an aliquot of plant extract and the standard drugs at varying concentrations (0.025–0.400 mg/mL). The mixture was incubated for 2.5 h at 27 °C. The incubated mixture was added to Griess reagent (0.33% sulphanilamide dissolved in 20% glacial acetic acid and added to 0.1% w/v of 1-naphthylethylenediamine in ratio 1:1) in ratio 1:1 and incubated for 30 min at room temperature. At absorbance of 540 nm, the mixture was measured against the blank (Methanol) and percentage scavenging of samples was estimated with the equation;

$$\% \text{ NO scavenging} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

The samples scavenging activities providing IC₅₀ was calculated by plotting percentage scavenging of NO of the samples against the corresponding sample's concentration.

2.7. Statistical analysis

The experiments were done in triplicates and the mean value of results was expressed as a mean ± SEM. The data were subjected to statistical one-way analysis of variance and samples differences were extrapolated by Duncan's multiple range test, where it is applicable. Mean values of results were considered statistically significant when *P* < 0.05.

3. Results

3.1. Phytochemicals

After the plant samples were macerated, filtrated and dried, the extract yield of all the solvents for the leaf was found to be in the order of: water > methanol > ethanol > acetone extracts. Whereas, in the root extraction, it was in the order of: methanol > water > ethanol > acetone extracts. Among the different solvent extractions used in the study, water was found to have higher recovery content over other solvent extraction of the leaf while the methanol extraction was higher than other solvent extracts of the root.

The total phenolic, flavonoid contents, proanthocyanidin contents, and flavonol of different extractions, were analyzed and presented in Table 1. Acetone extract of the sample's root gave the highest value of total phenolic content [(240.68 ± 3.50) mg GAE/g] while acetone extract of the sample's leaf had the highest content of total flavonoid and flavonol [(526.23 ± 17.52) mg QE/g and (558.25 ± 12.53) mg QE/g respectively]. The highest content of total proanthocyanidin was (645.38 ± 1.33) mg CE/g which was in the acetone extract of the root as shown in Table 1. Alkaloid and saponin were also present in the root and leaf extracts with significant amounts (*P* < 0.05). A quantitative estimate of the alkaloid and saponin content indicated that the leaf extract had higher alkaloid (4.78% ± 1.52%) and saponin (9.86% ± 0.24%) than that of the root with alkaloid (2.94% ± 1.09%) and saponin (8.22% ± 0.23%).

Table 1

Total proanthocyanidin, flavonol, flavonoid, and phenolic contents of *R. crispus* (mean ± SD, n = 3).

Samples	Phenolic (mg GAE/g)		Flavonoids (mg QE/g)		Flavonols (mg QE/g)		Proanthocyanidin (mg CE/g)	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
MEE	103.54 ± 3.70 ^d	144.53 ± 11.18 ^c	130.83 ± 5.47 ^f	197.59 ± 20.0 ^c	62.79 ± 3.50 ^d	36.30 ± 0.96 ^{c,f}	149.49 ± 0.89 ^d	275.38 ± 0.44 ^c
ETE	16.95 ± 12.03 ^f	211.71 ± 9.65 ^b	244.06 ± 10.83 ^d	276.31 ± 5.47 ^c	327.76 ± 9.43 ^b	45.19 ± 1.44 ^e	153.08 ± 0.0 ^d	421.02 ± 6.22 ^b
ACE	34.82 ± 17.11 ^f	240.68 ± 3.50 ^a	526.23 ± 17.52 ^a	371.95 ± 4.90 ^b	558.25 ± 12.53 ^a	83.03 ± 0.94 ^c	300.77 ± 14.21 ^c	645.38 ± 1.33 ^a
WAE	18.49 ± 0.92 ^f	54.85 ± 14.39 ^e	19.39 ± 4.08 ^h	89.09 ± 2.81 ^g	19.24 ± 5.99 ^{f,g}	15.24 ± 0.27 ^g	21.80 ± 0.89 ^e	176.15 ± 13.76 ^d

Means with different letter are significantly different (a > b > c > d > e > f > g > h). CE: Catechin equivalent; QE: Quercetin equivalents; GAE: Gallic acid equivalents.

3.2. Antioxidant activity

3.2.1. DPPH

Percentage inhibition of DPPH radical of the tested samples was in the order of: gallic acid > ACE of root > ETE of root > MEE of root > MEE of leaf > WAE of root > rutin > ACE of leaf > BHT > ETE of leaf > WAE of leaf (Figure 1). The results of series of sample's concentrations tested were used to determine the required concentration to attain fifty percent radical scavenging effect (IC₅₀) as shown in Table 2. It was observed that the value of IC₅₀ of a sample was inversely proportional to its scavenging activity. Significant antioxidant ability was shown by the acetone extract of the root of *R. crispus* at 50% inhibitory concentration (0.014 mg/mL), and the value was significant (P < 0.05) compare to gallic acid (0.005 mg/mL). The results showed that acetone extract of the root had better hydrogen donating ability compared to other extracts.

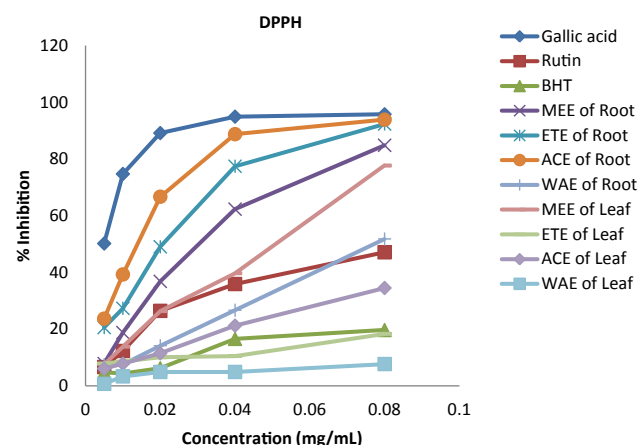


Figure 1. DPPH radical scavenging of *R. crispus*. MEE: Methanol extract; ETE: Ethanol extract; ACE: Acetone extract; WAE: Water extract.

3.2.2. ABTS

The IC₅₀ scavenging of ABTS of samples had a peak capacity in ACE of the root (<0.005 mg/mL) and gallic acid (<0.005 mg/mL) for the standard drugs (Table 2). The ABTS scavenging activity of samples decreased with increasing concentration (Figure 2). The scavenging power of tested samples was in the order of: gallic acid > ACE of root > ETE of root > MEE of root > MEE of leaf > WAE of root > ACE of leaf > WAE of leaf > BHT > rutin > ETE of leaf. The root of the plant showed a higher scavenging activity compare to the leaf and the standards except for the reference drug, gallic acid.

3.2.3. Phosphomolybdenum

The TAC of the sample was as a result of the formation of phosphomolybdenum complex which showed there was a rapid increase of antioxidant activity as concentration increases. The percentage inhibition of total antioxidant of tested samples was

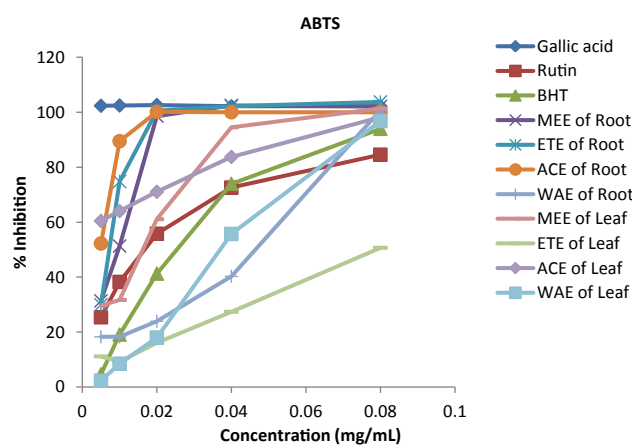


Figure 2. ABTS⁺ radical scavenging activity of *R. crispus*. MEE: Methanol extract; ACE: Acetone extract; ETE: Ethanol extract; WAE: Water extract.

Table 2

IC₅₀ of *R. crispus* extracts in ABTS, FRAP, DPPH, NO and phosphomolybdenum assays (mg/mL).

Samples	ABTS		FRAP		Phosphomolybdenum		DPPH		NO	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
MEE	0.016 0	0.009 7	0.027 0	0.168	0.218 0	0.128 0	0.050 0	0.030 0	0.163 0	0.133 0
ETE	0.078 0	0.007 3	0.0320	0.170	0.228 0	0.115 0	>0.080 0	0.020 0	>0.400 0	0.106 0
ACE	>0.025 0	<0.005 0	>0.400 0	0.075	0.109 0	0.048 0	>0.080 0	0.014 0	>0.400 0	0.067 0
WAE	0.037 0	0.046 0	>0.400 0	0.127	>0.400 0	0.174 0	>0.080 0	0.077 0	>0.400 0	0.233 0
Ascorbic acid	–	–	–	–	0.028 0	–	–	–	–	–
Gallic acid	<0.005 0	–	0.219 0	–	0.041 0	–	0.005 0	–	0.034 0	–
Rutin	0.016 6	–	0.086 0	–	0.138 0	–	>0.080 0	–	0.077 0	–
BHT	0.025 0	–	0.059 0	–	0.051 0	–	>0.080 0	–	0.137 0	–

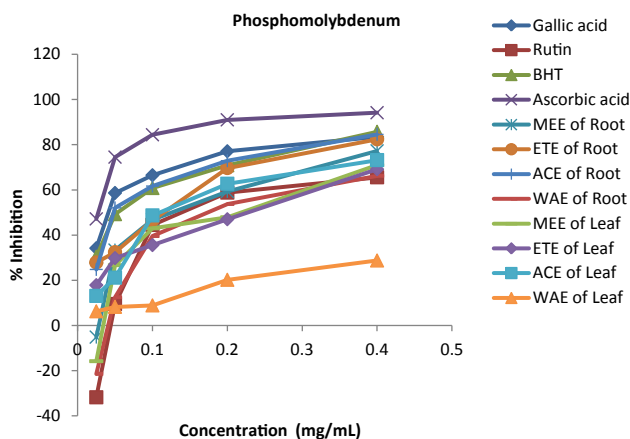


Figure 3. TAC of *R. crispus*.

MEE: Methanol extract; ETE: Ethanol extract; ACE: Acetone extract; WAE: Water extract.

in the order; ascorbic acid > gallic acid > ACE of root > BHT > ETE of root > MEE of root > ACE of leaf > MEE of leaf > ETE of leaf > WAE of root > rutin > WAE of leaf (Figure 3). The result of phosphomolybdenum complex activity was used to determine the required concentration that attain 50% radical scavenging effect (IC_{50}) as shown in Table 2. Ascorbic acid had the highest IC_{50} among the standard and acetone extract of the root had the highest among plant extract with value 0.028 mg/mL and 0.048 mg/mL, respectively. Percentage inhibition of the samples at the least concentration (0.025 mg/mL) was negative for rutin, methanol extract of root, water extract of root and methanol extract of leaf. This could be as a result of insufficient formation of phosphomolybdenum complex.

3.2.4. FRAP

The FRAP was determined by the ferric reducing ability of the standards and plant extracts (Table 2). It was recorded that, increase in absorbance of the reaction mixture is an indication of an increase in reducing power [22]. In this study, the reducing power of the plant extracts and standards correlated with their concentrations and it was in the order: gallic acid > rutin > BHT > ACE of root > ETE of root > MEE of leaf > MEE of root > ETE of leaf > WAE of root > ACE of leaf > WAE of the leaf as shown in Figure 4. FRAP of the plant extracts was less than the standard but was significant ($P < 0.05$). As shown

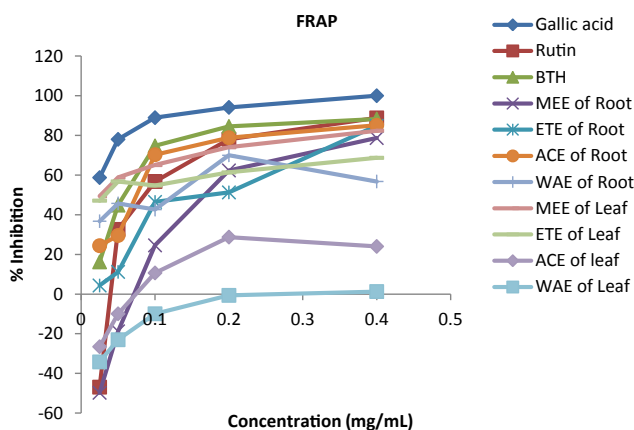


Figure 4. Ferric reducing antioxidant power of *R. crispus*.

MEE: Methanol extract; ETE: Ethanol extract; ACE: Acetone extract; WAE: Water extract.

in Figure 4, rutin, the methanol extract of root, acetone extract of leaf and water extract of leaf exhibited weak ferric reducing power and hence gradually increased from negative to positive as concentration increases.

3.2.5. NO scavenging activity

A decrease in the value of absorbance of the mixture was an increase in NO scavenging activity which indicated that samples are dose dependent. NO scavenging ability of the samples based on percentage inhibited was in the order: rutin > MEE of the root > WAE of the root > ETE of the root > ACE of the root > gallic acid > MEE of the leaf > BHT > WAE of the leaf > ACE of leaf > ETE of the leaf (Figure 5). The IC_{50} of ACE of the root had the lowest value (0.067 mg/mL), followed by ETE of root (0.106 mg/mL) which was an indication of strong NO scavenging activity with minimum concentration to attain 50% radical scavenging (Table 2).

The mean antioxidant activity of root extract was higher compared to extracts of the leaf samples. The activity difference obtained from root and leaf samples might be due to the photochemical constituent, extraction procedures and samples processing. This may also be the justification for frequent use of the root regularly in folklore medicine to treat helminthic infections.

4. Discussion

Plants are the source of phytochemicals and possess several biological activities. Functional property of a plant relies upon the different secondary metabolites it possesses such as: phenolics, terpenoids, or alkaloids [20]. The phytochemicals in plants greatly determined the antioxidant, antimicrobial and anti-inflammatory capacity. This capacity is primarily due to their redox properties [23] which are important in adsorbing and scavenging of free radicals [24]. It is, therefore, justifiable to evaluate the polyphenols content in the plant. Among the phytochemicals, polyphenols and flavonoids have been proven to be of great importance because they help the human body to fight against diseases. Flavonoids act as potent antioxidants but depend on their molecular structures and the position of the hydroxyl group in its chemical structure [6]. Results obtained in this study shows that the level of polyphenol

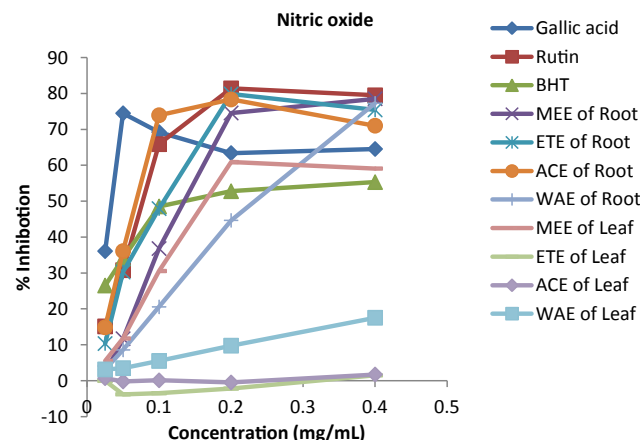


Figure 5. NO scavenging activity of *R. crispus*.

MEE: Methanol extract; ETE: Ethanol extract; ACE: Acetone extract; WAE: Water extract.

compounds evaluated in the extracts of the leaf and root of *R. crispus* was relatively significant.

Percentage yield of the extract of the leaf is highest in water followed by methanol extract, whereas the percentage yield of the root extract was highest in the methanol extract of the root, followed by water extract. This could be due to a closer polarity of water and methanol and the yield of the solvents seems to be inverse to the polarity. Acetone extract of the root and leaf of *R. crispus* gives a higher value of phenolic, flavonoid, flavonols and proanthocyanidin. Acetone is less polar and the only polar aprotic solvent used during extraction which could be the reason it has higher values of phytochemicals in its extract compare to other solvent used. Phenols, flavonoids and flavonols are polyphenolic compounds of plants which bring about substantial antioxidant activity and several biological activities including: anthelmintic, analgesic, anti-inflammatory, antimicrobial and anti-allergic properties [13,25]. These compounds have been described in several studies as free radicals scavengers against lipid peroxyl, superoxide anion, hydroxyl radicals and several health-promoting functions [18]. The oxidative capacity of *R. crispus* extracts could be due to the presence of phytochemicals. Therefore, it can be concluded that the phytochemicals present in *R. crispus* might have a synergistic effect and this could make it medicinally important rather than being poisonous.

The radical scavenging ability and antioxidant properties of plants are associated with its phytochemical constituents [25]. In this study, the antioxidant activity of *R. crispus* was measured using the following assays: FRAP, DPPH, ABTS, phosphomolybdenum and NO. Using a single assay to estimate the antioxidant properties of a sample will be insufficient to correctly estimate the antioxidant activity because it is influenced by many factors [6]. It is therefore important to carry out more than one type of antioxidant assay to cover the different mechanisms of antioxidant action.

The relatively high radical scavenging of stable DPPH by acetone extract of the leaf and root of the plant might be due to the high level of flavonoids which might account for such a strong activity. The average percentage inhibition of ABTS^{•+} by the plant extracts was found to be higher than that of DPPH. The value of IC₅₀ of ABTS is therefore quite lesser than that of DPPH. The different mechanisms involved in the radical-antioxidant reactions of the two assays might be the reason. This result validates the report of Oyedemi *et al.* [18] that some compounds might scavenge ABTS^{•+} but may not exhibit DPPH scavenging activity.

During the scavenging of NO free radical generated from sodium nitroprusside in the aqueous solution reacts with oxygen molecule to form nitrite. There are several studies that reported that NO is important in various inflammatory processes such as ulcerative colitis, sclerosis, carcinomas, diabetes, and arthritis [26–28]. The methanol extract of the root has higher percentage inhibition of NO followed by water extract of the root. But the acetone extract of the root has the highest IC₅₀ followed by ethanol extract of the root and this indicates that acetone extract of the root is very active at a very low concentration. It was observed that NO scavenging reduces with increase in concentration which could be due to the depletion of oxygen and NO by the sample. This conforms to the research of Oyedemi *et al.* [18], who identified that plant extracts exhibited NO radical scavenging by competing with oxygen, NO and its derivatives.

The result of this study shows the presence of alkaloids, phenols, flavonoids, saponin and proanthocyanidin in the solvents extract of the root and leaf of *R. crispus*. The root of the plant has more phytochemicals contents than the leaf and also showed a slightly higher antioxidant activity when compared with the leaf. The level of phenolic compounds and other phytochemicals in the plant must have contributed to its radical scavenging activity which aids its medicinal properties for the treatment of ailments. Thus, this may also justify the reason the root is more used in traditional medicine to treat helminthic infections. This is an ongoing study and further research is being carried to investigate the biological activities of the plant.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] Hlila MB, Mosbah H, Zanina N, Ben Nejma A, Ben Jannet H, Aouni M, et al. Characterisation of phenolic antioxidants in *Scabiosa arenaria* flowers by LC-ESI-MS/MS and NMR. *J Pharm Pharmacol* 2016; **68**(7): 932-40.
- [2] Kumarasingha R, Preston S, Yeo TC, Lim DS, Tu CL, Palombo EA, et al. Anthelmintic activity of selected ethnomedicinal plant extracts on parasitic stages of *Haemonchus contortus*. *Parasit Vectors* 2016; **9**(1): 187.
- [3] Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci* 2004; **74**(17): 2157-84.
- [4] Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem* 2004; **85**(2): 231-7.
- [5] Carvalho CO, Chagas AC, Cotinguiba F, Furlan M, Brito LG, Chaves FC, et al. The anthelmintic effect of plant extracts on *Haemonchus contortus* and *Strongyloides venezuelensis*. *Vet Parasitol* 2012; **183**(3): 260-8.
- [6] Iqbal E, Salim KA, Lim LB. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *J King Saud Univ Sci* 2015; **27**(3): 224-32.
- [7] Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Linnophila aromatica*. *J Food Drug Anal* 2014; **22**(3): 296-302.
- [8] Borneo R, León AE, Aguirre A, Ribotta P, Cantero JJ. Antioxidant capacity of medicinal plants from the Province of Córdoba (Argentina) and their *in vitro* testing in a model food system. *Food Chem* 2009; **112**(3): 664-70.
- [9] Lee SE, Hwang HJ, Ha JS, Jeong HS, Kim JH. Screening of medicinal plant extracts for antioxidant activity. *Life Sci* 2003; **73**(2): 167-79.
- [10] Wintola OA, Afolayan AJ. An inventory of indigenous plants used as anthelmintics in Amathole District Municipality of the Eastern Cape Province, South Africa. *Afr J Tradit Complement Alt Med* 2015; **12**(4): 112-21.
- [11] Grierson DS, Afolayan AJ. Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Africa. *J Ethnopharmacol* 1999; **66**(1): 103-6.
- [12] Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. *J Agric Food Chem* 2003; **51**(3): 609-14.
- [13] Oyedemi SO, Oyedemi BO, Arowosegbe S, Afolayan AJ. Phytochemicals analysis and medicinal potentials of hydroalcoholic extract from *Curtisia dentata* (Burm.f) CA Sm stem bark. *Int J Mol Sci* 2012; **13**(5): 6189-203.
- [14] Kumaran A, Karunakaran RJ. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT-Food Sci Technol* 2007; **40**(2): 344-52.

- [15] Unuofin JO, Otunola GA, Afolayan AJ. Phytochemical screening and evaluation of in vitro antioxidant and antimicrobial activities of *Kedrostis africana* (L.) Cogn. *Asian Pac J Trop Biomed* 2017; **7**(10): 901-8.
- [16] Otang WM, Grierson DS, Ndip RN. Phytochemical studies and antioxidant activity of two South African medicinal plants traditionally used for the management of opportunistic fungal infections in HIV/AIDS patients. *BMC Complement Alt Med* 2012; **12**(1): 43.
- [17] Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Glob J Pure Appl Sci* 2002; **8**(2): 203-8.
- [18] Oyedemi SO, Bradley G, Afolayan AJ. In-vitro and -vivo antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. *Afr J Pharm Pharmacol* 2010; **4**(2): 70-8.
- [19] Olugbami JO, Gbadegesin MA, Odunola OA. In vitro free radical scavenging and antioxidant properties of ethanol extract of *Terminalia glaucescens*. *Pharmacogn Res* 2015; **7**(1): 49.
- [20] Murugan R, Parimelazhagan T. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia* Arn. – an in vitro approach. *J King Saud Univ Sci* 2014; **26**(4): 267-75.
- [21] Jayanthi P, Lalitha P. Reducing power of the solvent extracts of *Eichhornia crassipes* (Mart.) Solms. *Int J Pharm Pharm Sci* 2011; **3**(3): 126-8.
- [22] Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antioxidant activities of methanol extract of *Sambucus ebulus* L. flower. *Pak J Bio Sci* 2009; **12**(5): 447.
- [23] Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem* 2001; **49**(11): 5165-70.
- [24] Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. *BMC Complement Alt Med* 2008; **8**(1): 53.
- [25] Alabri TH, Al Musalami AH, Hossain MA, Weli AM, Al-Riyami Q. Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. *J King Saud Univ-Sci* 2014; **26**(3): 237-43.
- [26] Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of *Spondias pinnata*. *BMC Complement Alt Med* 2008; **8**(1): 63.
- [27] Ksouri R, Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K, et al. Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. *Food Chem Toxicol* 2009; **47**(8): 2083-91.
- [28] Akinsanya B, Ade-Ademilua OE, Idris O, Ukwu UD, Saliu JK. Toxicological evaluation of plant crude extracts on helminth parasites of *Clarias gariepinus* using host low observed effect concentration (LOEC). *Egypt J Aquat Biol Fish* 2016; **20**(2): 69-77.