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Phytochemical and antioxidant activities of *Rumex crispus* L. in treatment of gastrointestinal helminths in Eastern Cape Province, South Africa

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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 (O_2^-) 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; 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, Na_2CO_3, $AlCl_3$, potassium acetate, Folin-Ciocalteu reagent, potassium iodide, acetone, ethanol, methanol, (H_2SO_4, ammonium solution, HCl, glacial acetic acid, NaCl, $K_3Fe(CN)_6$, 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 ciocalteau 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 ciocalteau reagent, and the mixture was diluted with distilled water in ratio 1:10 v/v and 2 mL of 7.5% w/v anhydrous $NaCO_3$ 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