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Antioxidant activity, total phenolic and total flavonoid contents of stem back and root methanolic extracts of *Calotropis procera*

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ABSTRACT

Calotropis procera it is known for traditional Kenyan medicinal system and it is used for it has previously been employed for treatment various diseases. This study aimed at examining methanolic extract of *C. procera* to establish antioxidant potential *in vitro*, total phenolic and flavonoid contents in order to identify potential sources of new antioxidants in food and pharmaceutical formulations. A comprehensive assessment on the antioxidant activity of stem back and root of *C. procera* by *in vitro* chemical analyses. Total phenolic, total flavonoid contents and total yield of the samples were also estimated. Preliminary phytochemical tests were also carried out to establish the components of plant extracts. Results of the study revealed presence of saponins terpenoids, alkaloids, anthraquinones and steroids. The stem back extract had significantly higher amounts of total phenol and flavonoid contents (79.80 ± 3.79 mg GAE/g extract and 71.63±4. mg RTE/g extract, respectively) compared to root samples. We established correlation between total phenolic contents and EC_{50} values for DPPH free radical scavenging property and reducing power of extract, ABTS radical cation and phosphomolybdate. Taken together, this work demonstrated considerable protective effectiveness in *C. procera* stem back and root methanolic extracts that function as an antioxidizing agent due to their free radical scavenging activity.

Keywords: ABTS radical cation, antioxidants, *Calotropis procera*, DPPH, reducing power.

INTRODUCTION

Recently, the use of medicinal plants as a source of naturally occurring, has considerably increased to replace synthetic antioxidants, which are being controlled owing to their ability to promotes carcinogenesis [1]. Among the bioactive compounds of medicinal plants, polyphenols, that perform a critical role in scavenging of free radicals/reactive oxygen species (ROS), acting on specific molecular targets and thus playing a vital role in human nutrition and health [2]. Some of these phytochemicals possess substantial antioxidant capacities and curtail the risk of several chronic human diseases such as cancer, arteriosclerosis, cardiovascular diseases, arthritis, neurodegenerative diseases, diabetes mellitus type 2 and Alzheimer's disease and aging, whose reduction is mainly related with strong antioxidant properties of phytochemicals [3, 4]. On the other hand, antioxidants (free radical scavengers) perform a critical role in health maintenance, and preventing diseases which are caused by oxidative stress [5]. Abnormal high levels of free radicals which overwhelm the antioxidant defense mechanisms result in oxidative stress which consequently results into cellular metabolism disturbances, regulation of metabolism and eventually cell damage [6]. The naturally occurring antioxidants may be inefficient hence intake of foods high in antioxidants is indispensable.

Plant phytochemicals that contain antioxidant compounds responsible for antioxidant activity can now be isolated and used as antioxidant therapy for the deterrence and treatment of free radical reactive oxygen species ROS-related diseases [7, 8]. Identification of natural antioxidant compounds in medicinal plants is therefore imperative. Even though it is unclear the compounds of medical plants which are the bioactive ones, polyphenols have recently been received growing interest because of interesting findings concerning their bioactivities. Antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid peroxidation, are the most important pharmacologically and therapeutically. Although it is known that a variety of medicinal plants and herbs are sources of phenolic compounds, polyphenol isolation studies and evaluation of their antioxidant effects have rarely been performed.

C. procera are the is most species reported in literature in the *Calotropis* genus. They both are used in traditional medicine, though both are considered as weeds. The plant has several pharmacological uses. Skin diseases are treated by the use of the whole plant. The bark of both plants is used for spleen complaints, ringworms, protracted labour, leprosy, dysentery, and syphilis. The latex has traditionally been used to treat and manage toothache, tumours, syphilis, leprosy, as well as an antiseptic and also for poisoning arrows. Flowers are used against respiratory complaints. Additionally, leaves are used as painkillers and for fever management (Kumar *et al*., 2017; Kumar *et al*., 2013; Singh *et al*., 2005). Because of their active elements and traditional applications, these plants are also regarded effective in the therapy and management of free radical related diseases. This study therefore investigated phenolic and flavonoid contents, antioxidant activity using various *in vitro* assays and phytochemistry of methanolic extracts from *C. procera.*

MATERIALS AND METHODS

Plant material

C. procera stem and roots were collected in December, 2017 from Mwingi, Kitui County, Kenya by the help of a local herbalist and transported in polythene bags to Biochemistry and Biotechnology laboratories at to Pwani university Science Laboratory for further processing and analysis. The plant specimens were botanically authenticated by a taxonomist at National Museums of Kenya and stored for future reference in the herbarium. Plant samples were shade dried at room temperature after washing under tap water to remove any debris. The pieces were then powdered using an electric mill, sieved through a mesh sieve. and kept in the dark until future use.

Phytochemical screening

Extraction of phenolics

Ground plant part material (20 g) was soaked in 150 ml methanol and shaken for 24 hours. Any methanol present was eliminated under reduced pressure in a rotary evaporator at 40 °C before twice removing fats the resulting the remaining aqueous extraction solution with petroleum ether to get rid of any present lipids. Subsequently, a lyophilized solution was acquired by using ethyl acetate in the presence of aqueous solution comprising 20% ammonium sulphate and 2% meta-phosphoric acid solution. Sufficient amount of anhydrous sodium sulphate was then added to the ethyl acetate fraction and then evaporated to dryness. The resulting precipitate was dried before dissolving it in 5 ml of absolute methanol and maintained at -20 ˚C for further analysis.

Determination of total phenolic contents

The amount of total phenolic compounds was analysed using Folin–Ciocalteu reagent [1]. Using gallic acid as the standard with concentrations ranging from 4 µM to 0.5 mM, a standard plot was generated. Firstly, 200 µl of 10% (v/v) Folin–Ciocalteu reagent was added to 100 µl of each extract in phosphate buffer (75 mM, pH 7.0). Thereafter, of 800 µl of 700 mM sodium carbonate (Na_2CO_3) was added and the reaction incubated in darkroom for 2 hours at room temperature. Gallic acid was used as positive control whereas phosphate buffer was used negative control. The absorbance was determined at 765 nm. Total phenolic compounds were expressed as equivalents of gallic acid per gram of dry extract weight ($mg_{GAE}/g_{extract}$).

Analysis of total flavonoid contents

Total flavonoid contents in the plant extracts was determined spectrophotometrically using the aluminium chloride colorimetric procedure [10]. Exactly 0.5 ml of the sample was mixed with 0.5 ml of 2% aluminium chloride ethanolic solution and incubated for at ambient temperature for 1 hour. Thereafter, the absorbance was measured at 420 nm and quercetin was used to create the standard curve. Total flavonoid contents were expressed as quercetin equivalent/g of sample (mgQE/g).

Text for presence of saponins

Two procedures were used to screen for existence of saponins in the *C. procera* extract. First, saponins ability to produce emulsion with oil was used as prescribed by Harborne (1973) with some modifications. Briefly, 20 mg of extract was boiled in 20 ml water for 5 minutes and filtered. About 10 ml of the filtrate was added to 5 ml of water and dynamically shaken to form froth. Thereafter, about three drops of olive oil were added to the froth and vigorously shaken. Observations were made for formation of emulsions. Secondly, about 2 ml of extract mixed with 2 ml of water and the resulting mixture was shaken well for 15 minutes then observed for development of foam.

Text for terpenoids

Terpenoids presence in the *C. procera* root and stem back extracts was carried out by adding 2 ml of chloroform to 5 ml (1 mg/ml) of the plant extract, followed by addition of 3 ml of conc. sulfuric acid. A reddish-brown interface colour verified terpenoid existence [12].

Text for presence of tannins

About 50 mg of the plant extract was boiled in 20 ml double distilled water and filtered. Several drops of 0.1% ferric chloride ($FeCl_3$) was added to the filtrate. Observation was made for any colour change. A brownish-green or a blue-black colour denoted for the presence of tannins [12].

Test for alkaloids

About 0.4 g of methanolic extract was mixed with 8 ml of 1% hydrochloric acid and the solution was briefly warmed for about 5 minutes and filtered. Exactly 2 ml of filtrate was reacted separately with first several drops of potassium mercuric iodide (Mayer’s reagent) and secondly potassium bismuth (Dragendroff’s reagent). Turbidity (white or creamy precipitate) in the first test and formation of a reddish-brown precipitate in the second test was taken as evidence for the presence of alkaloids in the plant extract.

Test for anthraquinones

Approximately 0.5 g of plant methanolic extracts were boiled with together with 6 ml of 1% hydrochloric acid and the hot solution mixture was filtered. About 5 ml of benzene was added to the filtrate and before shaking the solution gently. About 2 ml of 10% ammonia solution was further added to the resulting solution and the mixture was shaken gently. In the ammonia phase of the mixture, a purple, purple or red colour that developed confirmed presence free hydroxyl anthraquinones.

Test for steroids

To test for steroids, approximately 0.5 g of extract was dissolved in 2 ml chloroform. To the sides of the test tube, exactly 3 ml of conc. sulphuric acid before shaking the mixture gently. Presence of steroids in the plant extracts was confirmed by turning red of the upper layer (chloroform layer) and a yellow colour with a green fluorescence in the sulphuric acid (lower layer).

Evaluation of the antioxidant activity

DPPH radical scavenging assay

The ability of extracts to reduce radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was evaluated using the technique outlined by Masuda *et al.* [13] with some modifications. Exactly 50 µl of each extract at different concentrations (1 – 10 mg/ml) was added to 3 ml of DPPH methanol solution (0.1mM) and the solution was put at room temperature in the

dark for 30 minutes. Absorbance of a blank sample (methanol solution) at 517 nm was measured. We used butylated hydroxytoluene and quercetin as positive controls. The antioxidant activity was assessed by the plant extract concentration which neutralized fifty per cent of DPPH radicals (IC_{50} value).

ABTS radical cation decolouration assay

Potassium persulfate was used to prepare the 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS) and the stock solution (7 mM) and the mixture stored at ambient temperature for 12–16 hours in the darkroom [14]. Subsequently, 10 µl of each plant extract, trolox concentration (standard) or buffer (blank) were added to 190 µl $ABTS^{•+}$ solution in a clear 96-well microplate. Absorbance of the solution was assessed at 734 nm, exactly 1 minute after the initial mixing. The capacity of the test plant extract sample to scavenge ABTS radical cation was correlated to trolox standard and expressed as µmol trolox equivalents per gram of extract ($\mu mol_{TE}/g_{extract}$) for the sample dilution that caused 20–80% inhibition of blank absorbance.

$Fe^{3+}/Fe2^{+}$ reducing power assay

The ferric reduction ability of different plant extracts was evaluated by the method of Pulido *et al.* [15] with some slight changes. Dissimilar concentrations of both the stem back and root plant samples (2 ml) were mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide (10 mg/ml). The ensuing mixture was incubated 20 minutes at 50°C for after which 2 ml of trichloroacetic acid (100 mg / l) was added. The was centrifuged for 10 minutes at 3000 rpm and the upper layer of the solution that formed was carefully collected. Exactly 2 ml from each of the solution mixture previously stated was added to a mixture of 2 ml of distilled water and 0.4 ml of 0.1% (w/v) fresh ferric chloride. After 10 minutes reaction incubation at room temperature, the absorbance was measured at 700 nm. Ascorbic acid was used as the control. A higher reducing power of the reaction mixture revealed by higher absorbance values.

Phosphomolybdate antioxidant assay

The total antioxidant activity of *C. procera* methanolic stem back and root extracts was also estimated by the phosphomolybdenum method [16]. About 0.1 ml of each plant extract sample was added to 1 ml of reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate). The tubes containing the mixture were covered with silver foil and incubated for 90 minutes in a water bath at 95°C, before allowing the mixture was allowed to cool at room temperature. Absorbance of the solution was determined at 765 nm against a blank. Ascorbic acid was used as standard. Higher absorbance values indicated the higher total antioxidant potential of the plant extracts.

Statistical analysis

All the experiments were carried out in triplicate and were repeated three times. Collected data was analyed using one-way analysis of variance (AVONA), followed by Fisher's least significant difference (LCD) test to determine differences between the means. A value of at confidence level of 95% ($p \leq 0.05$) was treated to be statistically significant. Minitab 17 statistical software was used for data analysis. All graphs were drawn using SigmaPlot software. The results were presented as the means ± standard deviation (SD).

RESULTS

Analysis total phenolic and flavonoid contents

The results as shown in Table 1 revealed that the methanolic extracts of *C. procera* stem back and root had dissimilar content values with stem back having significantly higher total phenolic content of 79.80 ± 3.79 mg GAE/g while the root samples recorded as 70.64 ± 2.65 mg GAE/g of extract. Results for total flavonoid content for stem back and of C. procera are 71.63±4.26 and 43.09 ± 3.51 mg QE/g, respectively (Table 1), signifying that stem back had higher flavonoid contents than the roots.

Table 1: Total phenolics and flavonoid contents of *C. procera*

Plant material	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Stem back	79.80 ± 3.79	71.63±4.26
Root	70.64 ± 2.65	43.09 ± 3.51

Phytochemical screening

Phytochemical screening of *C. procera* methanolic extracts established the existence of saponins terpenoids, alkaloids, anthraquinones and steroids in the stem back and root methanolic extracts of *C. procera*. We did not detect the presence of tannins and cardiac glycosides.

In vitro antioxidant activity

DPPH radical scavenging assay

Fig. 1 shows the free radical scavenging potentials of *C. procera* stem back and root methanolic extracts at different concentrations (1–10mg/mL) measured by the DPPH radical scavenging assay. The degree of discoloration displayed the extracts' scavenging potential. Significant scavenging potential ranging from 34.86 to 80.71% was reported at reduced concentration of up to 4 mg / ml of both extracts. Any increase in concentration of the extracts did not yield any significant activity change for both extracts. At a lower concentration (0.5mg / mL), both BHA and BHT exhibited appreciable radical scavenging operations (about 95 percent). Overall, methanolic extracts from Kenyan *C. procera* species reached 90.55% DPPH inhibition at higher concentrations (10 mg/ml) in the present work. Our results therefore showed considerably greater higher antioxidant activity.

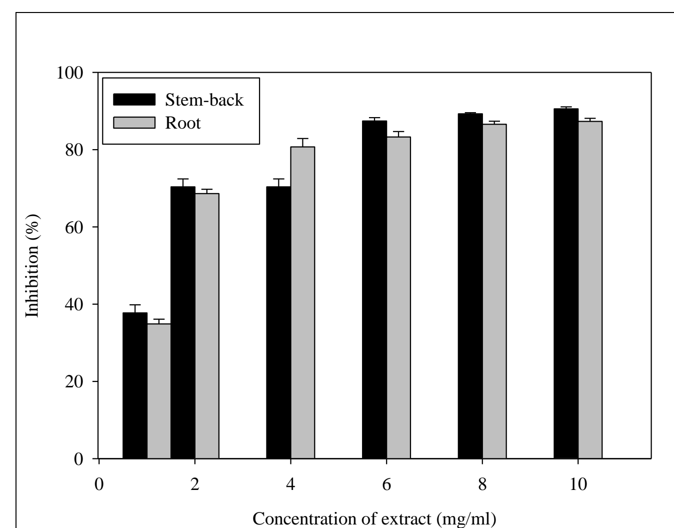

Figure 1: Free radical scavenging activity of *C. procera* stem-back and root methanolic extracts by DPPH assay.

ABTS radical cation decolouration assay

Both the stem back extracts of *C. procera* scavenged ABTS radical cation in a concentration-dependent manner (0 -250 µg/ml; Figure 2). Results from the study demonstrated that stem back samples had significantly higher ABTS radical scavenging ability than the root extracts. Both samples displayed prominent ABTS radical scavenging activities. The trolox equivalent antioxidant concentration value was 0.78 ± 0.02.

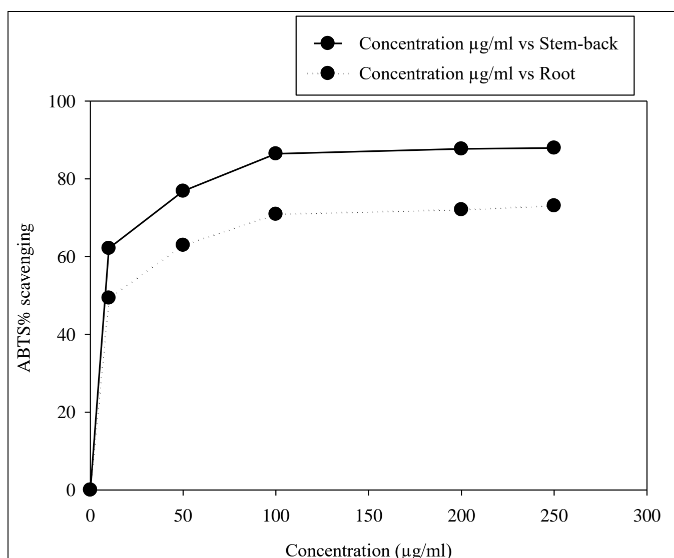

Figure 2: ABTS radical scavenging activity activities of stem back and root methanolic extracts of *C. procera* at various concentrations.

$Fe^{3+}/Fe2^{+}$ reducing power assay

Both the stem back and the root samples of *C. procera* recorded considerable reducing power ability, and results are shown in Figure 3. High absorbance values of the extract samples indicated increase reducing power capacity and increased reducing powers of the *C. procera* plant methanolic extracts signposted a strong antioxidant capacity. We also established that the reducing ability and antioxidant capacity of both stem back and root methanolic extracts and increased with increasing the concentration of extract displaying dose dependent response. Stem back fraction had significantly higher reducing power than the root extract.

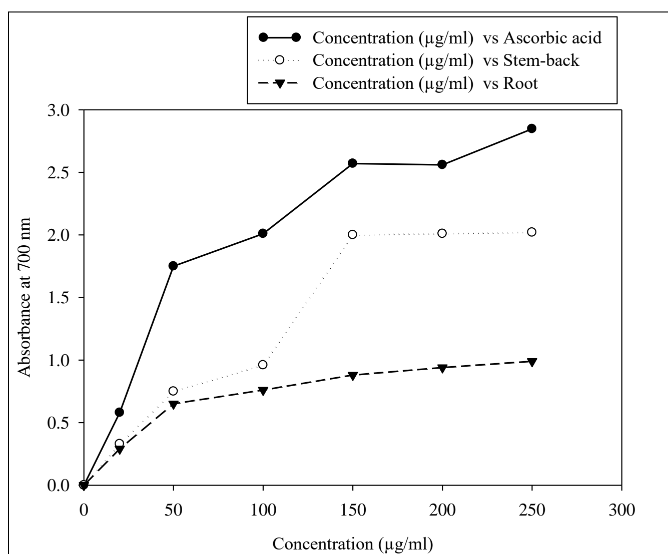

Figure 3: Reducing power of stem back and root methanolic extracts of *C. procera* at different concentrations. Each value represents a mean ± SD (n = 3).

Phosphomolybdate assay

The phosphomolybdate method is quantitative, as the total antioxidant capacity (TAC) is expressed as equivalent to ascorbic acid. The stem back extract was found to have more antioxidant capacity than the root fraction (Figure 4). The results of phosphomolybdate assay also showed antioxidant activity in dose-dependent manner at 25 to 250 µg/ml concentration levels. The EC_{50} value of antioxidant potential of stem back sample (13.38±72 µg/ml) was most pronounced ($P < 0.05$) than root sample (116±85 µg/ml). The strong antioxidant activity of stem back extract which was statistically analogous to that of ascorbic acid reveals strong antioxidants in this sample and this could be associated to presence of the high amounts of phenolic compounds present.

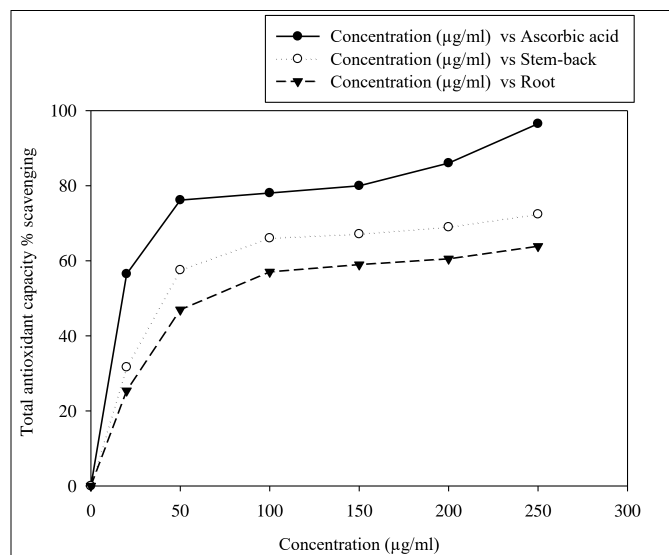

Figure 4: Total antioxidant capacity (TAC) of stem back and root methanolic extracts of *C. procera* at different concentrations.

DISCUSSION

In living organisms, normal cellular metabolism produces free radicals (any molecular species that is has the ability of independent existence and contains an unpaired electron in an atomic orbital). Because of their unpaired electrons, most of free radicals extremely reactive and unstable [16, 17]. Free radicals are associated with numerous pathological conditions such as diabetes, neurodegenerative diseases, cataracts, cancer, cardiovascular diseases, asthma, inflammation, rheumatoid arthritis, burns, intestinal tract diseases, progerias and ischemic and post-ischemic pathologies [17]. The most important oxygen-containing free radicals in many of these diseases are oxygen singlet, nitric oxide radical, hypochlorite, superoxide anion radical, hydroxyl radical, hydrogen peroxide, and peroxynitrite radical. Although there are several enzymes and nonenzymatic systems within the body that scavenge free radicals, phytochemicals with contain antioxidants that act as radical scavengers [18]. Phytochemicals have the ability to modify metabolism of humans in a way that is advantageous for the prevention, treatment and management chronic and degenerative ailments. Many synthetic drugs safeguard against oxidative damage but have side effects that are adverse and irreversible. As an alternative, consuming natural antioxidants from food supplements. A substitute solution to this challenge is the consumption of natural antioxidants from food supplements and herbal medicines. In this study, antioxidant properties of *C. procera,* a traditional medicinal plant that is widely used to treat several diseases in Kenya was studied.

Among the phytochemicals, phenolic compounds, ubiquitous in plants are crucial components of the human diet and have been reported to exert antioxidants activities in biological systems, acting as scavengers of reactive free radicals [19]. In addition, flavonoids are the largest group of compounds of plant phenolics, representing more than half of the thousands of natural phenolic compounds [20]. The study established that considerable amounts of phenolics and flavonoids were present in *C. procera* in both the stem-back and root methanolic extracts. Phytochemical screening tests of plant extracts showed the existence of glycoside linked tannins or flavonoids which further validating that most of the activities observed during this study were as a result of the presence of phenolics and flavonoids [20]. We found out that the stem back samples had significantly higher amounts of phenolics and flavonoids.

DPPH radical scavenging assay uses the electron donation ability of plant compounds and which is quantified by the bleaching of the purple

coloured solution by DPPH radical. The degree of change in colour is proportional to the potency concentration of the antioxidants. The high antioxidant properties observed in both stem-back and root extracts could be attributed to the presence of high quantities of phytochemical compounds (phenolic hydroxyls) capable of providing free radicals hydrogen to scavenge the potential change. Also, a significant decrease in the absorbance of the reaction mixture demonstrates the compound's significant free radical scavenging activity under test [21]. This study found out that the stem-back methanolic extracts of C. procera had significantly higher inhibition percentage and which positively correlated with total phenolic content when compared with extract from the roots. These results suggest that the plant extract contains phytochemical components capable of giving hydrogen to a free radical in order to scavenge the potential damage.

ABTS radical cation decolorization assay is a spectrophotometric diode-array technique used for colour evaluation loss when adding an antioxidant to ABTS* (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) blue-green chromophore. The antioxidant reduces and decolorates ABTS* to ABTS. ABTS test is therefore an attractive tool for evaluating the antioxidant activity of hydrogen donation and antioxidant chain breaking. Trolox, an analogous water-soluble vitamin E, acts as a positive control that inhibits dose-dependent creation of the radical cation. Miliauskas *et al*., [22] evaluated the antioxidant activity of commonly used medicinal plants and herbs and confirmed that significant ABTS scavengers are phenolic compounds. Results from this research revealed that methanolic extracts of *C. procera* stem back and root reduced ABTS* and this significantly antioxidant effect could be ascribed to the presence of phenolics compounds. Stem back samples exhibited higher ABTS* reducing capacity when compared root extract. Furthermore, since the extracts from different techniques of extraction have the capacity to scavenge free radicals, thus stopping lipid oxidation through a chain-breaking response, when ingested along with nutrients, they could serve as prospective nutraceuticals.

The easy and dependable FRAP assay has been regularly to evaluate the reduction potential of a of an antioxidant reacting with a ferric TPTZ complex to produce a coloured ferrous 2,4,6-tripyridyl-s-triazine (TPTZ) complex [16]. Depending on the reduction capacity of the compound, the sample solution normally changes its colour from yellow to several shades of blue and green. The concentration of ferric ions can be easily calculated by measuring the formation of Pearl's Prussian Blue at 700 nm [23]. The reducing potential usually depends on the reductants present in a compound that exhibited antioxidant capacity by donating a hydrogen atom or breaking a free radical chain [24]. The reduction potential generally relies on the the presence of phenolic compounds that act by donating a hydrogen atom or breaking a free radical chain. Hence, *C. procera* phenolics and flavonoids are capable of disrupting the free radical chain. The reduction capacity of the reference compound (ascorbic acid) at the same levels was discovered to be better than the stem back extract. Our results also correlate with the findings of Fu *et al*., [25] and Do *et al*., [26].

A spectrophotometric assay was developed to quantitatively determine the total antioxidant capacity using the phosphomolybdenum. This technique is based on the reduction of molybdenum (IV) to molybdenum (V) by the plant sample and the ensuing production of phosphate/molybdenum (V) complex at acidic conditions, which is green in colour. The use of phosphomolybdate assay has been reported as an effective method to evaluate the total antioxidant potentials of various plant extracts [27, 28, 29]. In our study, the antioxidant capacity of both the stem back and root extracts suppressed Mo^{5+} complex. From this observation, the total antioxidant capacity of *C. procera* could be as a result of the presence of phenolics and flavonoids in the plant methanolic extracts.

CONCLUSION

Results from the *in vitro* assays assays of the present study established that both stem bark and root methanolic extracts *of C. procera* possess considerable antioxidant property as witnessed by the results of DPPH radical scavenging assay, ABTS radical cation decolouration assay, reducing power assay and phosphomolybdate antioxidant assay and could help prevent the progress of different oxidative stresses. This antioxidant potential of the extracts may be attributed to several phytochemicals present in the extract. The components accountable to the antioxidant activity, however, are unclear at the moment. In order to isolate and identify the antioxidant compounds present in the plant extracts, further investigation is therefore necessary. In addition, these extracts *in vivo* antioxidant activity must be evaluated prior to clinical use.

Conflict of interests

Author declare no conflict of interest.

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REFERENCES

1. Muddathir AM, Yamauchi K, Batubara I, Mohieldin EAM, Mitsunaga T. Anti-tyrosinase, total phenolic content and antioxidant activity of selected Sudanese medicinal plants. South African Journal of Botany. 2017; 109:9-15.
2. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. Journal of chromatography 2004; 1054(2):95-111.
3. Wojtunik-Kulesza KA, Oniszczuk A, Oniszczuk T, Waksmundzka-Hajnos M. The influence of common free radicals and antioxidants on development of Alzheimer's Disease. Biomedicine & pharmacotherapy. 2016; 78:39-49.
4. Maritim AC, Sanders A. Watkins JB. Diabetes, oxidative stress, and antioxidants: a review. Journal of biochemical and molecular toxicology. 2003; 17(1):24-38.
5. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. European journal of medicinal chemistry. 2015; 97:55-74.
6. Breitenbach M, Eckl P. Introduction to oxidative stress in biomedical and biological research. Biomolecules. 2015; 5:1169-1177.
7. Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological reviews. 2000; 52(4):673-751.
8. Packer L, Rimbach G, Virgili F. Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinus maritima*) bark, pycnogenol. Free radical biology and medicine. 1999; 27(5-6):704-724.
9. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature protocols. 2007; 2(4):875-877.
10. Woisky RG, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. Journal of apicultural research. 1998; 37(2):99-105.
11. Harborne JB. Phenolic compounds. In Phytochemical methods (1973); Springer, Dordrecht.
12. Iqbal Z, Sarwar M, Jabbar A, Ahmed S, Nisa M, Sajid MS, Khan MN, *et al*. Direct and indirect anthelmintic effects of condensed tannins in sheep. Veterinary parasitology. 2007; 144(2):125-131.
13. Masuda T, Yonemori S, Oyama Y, Takeda Y, Tanaka T, Andoh T, *et al*. Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. Journal of agricultural and food chemistry. 1999; 47(4):1749-1754.
14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine. 1999; 26(9-10):1231-1237.
15. Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of agricultural and food chemistry. 2000; 48(8):3396-3402.
16. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian journal of clinical biochemistry. 2015; 30(1):11-26.
17. Umamaheswari M, Chatterjee TK. *In vitro* antioxidant activities of the fractions *of Coccinia grandis* L. leaf extract. African journal of traditional, complementary and alternative medicines. 2008; 5(1):61-73.

18. Gutteridge JM, Halliwell B. Invited review free radicals in disease processes: a compilation of cause and consequence. Free radical research communications. 1993; 19(3):141-158.
19. Shahidi G, Venskutonis PR, van-Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food chemistry. 2004; 85(2):231-237.
20. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews. 2010; 4(8):118-126.
21. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. Journal of functional foods. 2015; 18:820-897.
22. Galati G, O'brien PJ. Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. Free radical biology and medicine. 2004; 37(3):287-303.
23. Krishnaiah D, Bono A, Sarbatly R, Anisuzzaman SM. Antioxidant activity and total phenolic content of an isolated *Morinda citrifolia* L. methanolic extract from Poly-ethersulphone (PES) membrane separator. Journal of King Saud University-engineering sciences. 2015; 27(1):63-67.
24. Miliauskas G, Venskutonis PR, van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food chemistry. 2004; 85(2):231-237.
25. Neto AO, Farias LA, Dias RR, Brandalise M, Linardi M, Spinacé EV. Enhanced electro-oxidation of ethanol using PtSn/CeO2–C electrocatalyst prepared by an alcohol-reduction process. Electrochemistry communications. 2008; 10(9):1315-1317.
26. Alam MN, Bristi NJ, Rafiquzzaman M. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi pharmaceutical journal. 2013; 21(2):143-152.
27. Fu ZF, Tu ZC, Zhang L, Wang H, Wen QH, Huang T. Antioxidant activities and polyphenols of sweet potato (*Ipomoea batatas* L.) leaves extracted with solvents of various polarities. Food bioscience. 2016; 15:11-18.
28. 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 *Limnophila aromatica*. Journal of food and drug analysis. 2014; 22(3):296-302.
29. Murugan R, Parimelazhagan T. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia* Arn.–An *in vitro* approach. Journal of King Saud University-science. 2014; 26(4):267-275.
30. Jan S, Khan MR, Rashid U, Bokhari J. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. Osong public health and research perspectives. 2013; (5):246-254.
31. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC complementary and alternative medicine. 2012; 12(1):221-233.

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