Effects of Cooking and Drying on the Total Phenolic, Total Flavonoid Content, Antioxidant and Antibacterial Activity of *Cleome gynandra* (Spider Plant)

Moyo S. M, Kayitesi E, Mavumengwana V and Madala N. E.

***Abstract*—** *Cleome gynandra* is an underutilised leafy vegetable that has a high nutritive value and contains phenolic compounds that are essential in reducing or preventing the occurrence of chronic and infectious diseases. Cooking and drying of the vegetable may induce changes to the vegetable's phenolic content, antioxidant and antibacterial activity. *C. gynandra* was subjected to boiling, steaming, blanching and drying processes and analysed for its Total Phenolic Content (TPC), Total Flavonoid Content (TFC), antioxidant and antimicrobial activity as well as Fourier transform infrared spectroscopy (FTIR). Cooking and drying significantly ($p < 0.05$) increased the levels of phenolic compounds and antioxidant activity in the vegetable. Antibacterial activity was generally negatively affected by cooking while drying improved the activity. Cooking and drying exerted positive effects on the vegetable's phenolic content and hence its importance in preventing and reducing chronic diseases is enhanced**.**

***Index Terms*—** *Cleome gynandra*, Cooking, Drying, Phenolic compounds.

I. Introduction

For the purposes of optimal health, ideal growth and development as well as prevention of disease, a good diet is essential [1]. As part of a good diet, Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) suggest at least 400 g of fruit and vegetable portion per day so as to preclude chronic diseases such as cancer, diabetes and heart diseases [2]. African leafy vegetables (ALVs) have been recognised as essential contributors of a balanced diet [3] as well as functional foods as they provide health beyond the basic nutritional requirements [4]. As such *Cleome gynandra* (spider plant) is an African green leafy vegetable that is consumed in various African countries such as Botswana [5], Ghana [6], Kenya [7], Namibia [8], South Africa [4], Tanzania [9] and Zimbabwe [10]. It has been utilised traditionally to treat stomach aches, headaches [11], ear diseases [12], food poisoning, rheumatism, sexual asthenia, antibacterial infections and snake bites [13]. Some studies have also shown its ability to act as an antioxidant [14], [15], [13], [16], [10], [4] while some have shown its antibacterial properties [17], [18]. Such health benefits could be attributed to its phenolic composition that has been reported in earlier studies [13],[19],[20],[21],[22],[23]. Despite these findings, the knowledge of the influence of cooking and drying on the plant is lacking. *C. gynandra* leaves are cooked before consumption or dried for preservation purposes and it has been established that these processes can exert negative or positive effects on vegetables in general [24], [25], [26], [27], [28], [29], [30]. The magnitude of each effect depends on the vegetable material as well as the cooking and drying conditions applied. Variations in the effects of cooking and drying could also occur due to different extraction methods, solvents used and type of assay employed.

According to [31], three basic reactions may induce changes on phenolic compound concentration during cooking i.e. oxidative reactions that may include enzymatic browning, formation of free phenolic compounds from their conjugate forms and conjugation of phenolic structures with related compounds. Processing of vegetables may also influence qualitative changes, antioxidant degradation and leaching of antioxidants into cooking water [27]. It is therefore necessary to probe the effects cooking and drying exert on *C. gynandra* as it contains remarkable health benefits linked to its phenolic composition. For that reason, this study aims to determine the total phenolic content, total flavonoid content, and antioxidant activity together with the antibacterial activities of *C. gynandra* after different cooking and drying conditions. Since phenolics possess hydroxyl and carboxyl groups [32] that are likely to be affected by cooking and drying, Fourier transform infrared spectroscopy (FTIR) was used to further assess the effects exerted upon the functional group presence in cooked and dried *C. gynandra* samples.

II. Procedure

A. Chemicals

All reagents used were of analytical or HPLC grade. Gallic acid, 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Quercetin, 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazil (DPPH) radical, Aluminium Chloride, Sodium acetate trihydrate, Sodium carbonate, Sodium hydroxide, Sodium nitrite, Acetic acid, Hydrochloric acid, Methanol, Folin-Ciocalteu reagent and 2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

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S. M. Moyo is with the Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg. P. O. Box 17011, Doornfontein Campus, Johannesburg, South Africa.

E. Kayitesi is with the Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, P. O. Box 17011, Doornfontein Campus, Johannesburg, South Africa.

V. Mavumengwana is with the Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, P. O. Box 17011, Doornfontein Campus, Johannesburg, South Africa.

N.E Madala is with the Department of Biochemistry, Faculty of Science, University of Johannesburg, P. O. Box 524, Auckland Park 2006, South Africa.

(ABTS) radical were purchased from Sigma–Aldrich (Sigma Chemical Co, Germany). Bacterial strains used were obtained from Davies diagnostics, (Randburg South Africa).

B. Sample preparation

Fresh *C. gynandra* leaves were harvested from the cultivating lands in the region of Venda of the Limpopo province South Africa. The leaves were washed thoroughly with tap water and drained to remove excess water. Thereafter the leaves were chopped and divided into six parts for different processing methods. The raw leaves were freeze dried.

Boiling: 200 g of leaves were added to 1 L of boiling water and cooked for 2 hours as it is a tough vegetable. The boiled leaves were removed from the boiling water and cooled. A sample of the water used to boil the leaves (boiled sample filtrate) was kept in the refrigerator for analysis and the leaves were freeze dried.

Steaming: 200 g of leaves were cooked for 2 hours in a steaming basket put above boiling water and covered. The leaves were then cooled and freeze dried.

Blanching: 200 g of leaves were immersed in a large pan of 1L boiling water for 5 minutes, cooled and freeze dried.

Drying: 200 g of leaves were dried to a constant weight in a conventional oven for about 8 hours at 50°C. A portion of the blanched leaves was also dried to a constant weight for about 8 hours at 50°C.

C. Solvent extraction

2g of powdered leaf material was mixed with 20 mL of 80% methanol and sonicated for 10 minutes. Thereafter the sonicated mixture was centrifuged for 10 minutes at 3000 rpm while maintaining a temperature of 4°C. The mixture was then evaporated to 1 mL using a rotary evaporator. The extracts were covered using perforated foil and left to dry in a cool dry area and stored at - 20°C before analysis.

D. Determination of Total Phenolic Content

The total phenolic content (TPC) of the extracts was approximated by Folin-Ciocalteau (F-C) method according to [33]. An iMark microplate absorbance reader (Bio-Rad laboratories 168 – 1130) was used to read absorbance at 750 nm and results expressed as mg Gallic acid equivalents/ 100 grams dry weight using the standard curve ($R^2 = 0.9902$).

E. Determination of Total Flavonoid Content

Total Flavonoid content (TFC) of the extracts was approximated by Aluminum chloride method according to [34]. Absorbance was read at 450 nm and results expressed as mg Quercetin equivalents/100 g dry weight using standard curve ($R^2= 0.9901$).

F. Determination of Antioxidant Activity

1) ABTS radical scavenging activity assay

The ABTS-2,2- azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) assay according to reference [35] was used to determine radical scavenging activity. Readings were measured at 750 nm and results expressed as µM Trolox Equivalent/g dry mass using standard curve ($R^2 = 0.9865$).

2) DPPH radical scavenging activity assay

he DPPH- (2,2-diphenly-1- picrylhydrazyl) assay was measured according to the method of reference [36]. Results were expressed as µM Trolox Equivalent/g dry weight using standard curve ($R^2 = 0.9893$)

3) Ferric reducing antioxidant power assay (FRAP)

The free radical scavenging assay was determined according to reference [37]. Results were expressed as µM Trolox Equivalent/g dry mass using standard curve ($R^2 = 0.9906$).

G. Determination of Antibacterial Activity (Minimum Inhibitory Concentration)

Minimum inhibitory concentration (MIC) according to reference [38] was used to determine the susceptibility of the following bacterial strains (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Enterococcus aerogenes* ATTC13048 *and Enterrococcus cloacae* ATCC13047) against the extracts of *C. gynandra*.

H. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrophotometer [Thermo Scientific Smart iTR, (Attenuated Total Reflectance), Thermo Fisher Scientific Inc. USA was used to obtain spectra for all cooked and dried samples. Spectrum for each sample was recorded in the region 4000 cm^{-1} – 550 cm^{-1} at 16 runs per scan as this is the characteristic peak range for phenolic compounds functional groups.

I. Statistical Analysis

The obtained results from all experimental methods were statistically analyzed by use of the SPSS statistical software (version 23). One-way Analysis of Variance (ANOVA) and Duncan's multiple range tests were used to analyze the means between the cooked and dry samples. A significant difference was considered when $P < 0.05$. The Pearson's correlation test was used to determine the relation between TPC, TFC and antioxidant activity. Means and standard deviations from the TPC, TFC, DPPH, ABTS and FRAP are results of two experiments performed in triplicate. The Unscrambler X statistical software version (10.4) was used to plot FTIR data.

III. Results and Discussion

A. Total Phenolic and Flavonoid Content.

Effects of cooking and drying on TPC are shown on table 1. TPC means among the cooked, dried and raw sample was found to be significantly different at $p< 0.05$ level. The boiled sample filtrate depicted the lowest TPC of 157.23 ± 0.67 mg GAE/100 g dw whereas the steamed sample showed the highest TPC of 3074 ± 81.9 mg GAE/100 g dw. By use of Duncan multiple comparison test, there exists significant differences between each processing method applied. The raw leaf extract exhibited a considerable amount of TPC of 812.01 ± 24 mg GAE/100 g. This result is lower than that obtained by [4] of 1923.9 mg GAE/100 g and higher than that obtained by [39] of 321.0 ± 2.65 mg GAE/100 g perhaps due to the varied environmental growing conditions that could affect phenolic compound composition present in plant as well as extraction method used.

The TFC of the raw, cooked and dried samples as portrayed in table 1 were found to be significantly different at $p < 0.05$. Boiled sample filtrate resulted in the lowest TFC of 15.85 ± 1.49 mg QE/100 g whereas the Blanched sample had the highest TFC of 446.11 ± 28.53 mg QE/100 g. The Duncan multiple range test shows that all sample's TFC means were significantly different from each other. The raw sample depicted a TFC of 71.55 ± 12 mg QE/100 g which is comparable to the results of [14] who recorded a value of 0.648 ± 0.25 mg QE/g.

TABLE I
EFFECT OF COOKING AND DRYING ON TPC AND TFC OF RAW *C. GYNANDRA* LEAVES.

Sample Type	TPC (mg GAE/100 g) *	TFC (mg QE/100 g) *
Raw	812.01 ± 24.1^{b}	71.55 ± 11.88^{b}
Boiled	906.40 ± 18.2^{c}	244.84 ± 14.64^{d}
Boiled sample filtrate	157.23 ± 0.67^{a}	15.85 ± 1.49^{a}
Blanched	1996.65 ± 87.5^{e}	446.11 ± 28.53^{g}
Dried	965.41 ± 53.07^{c}	150.24 ± 10.59^{c}
Blanched & dried	1357.01 ± 17.12^{d}	299.40 ± 40.75^{e}
Steamed	30 74.17 ± 81.9^{f}	375.09 ± 22.69^{f}

*Results expressed in dry matter weight (dw). TPC – Total Phenolic Content, TFC – Total Flavonoid Content, GAE- Gallic Acid Equivalent, QE- Quercetin Equivalent. All data reported as means ± SD (n=6). Alphabets in superscripts within a column show significant differences among the cooking and drying methods.

The analysis of the effects of cooking and drying on the TPC and TFC of the raw *C. gynandra* indicated that the levels of these compounds could be improved after cooking and drying. Boiling is the most common method of cooking *C. gynandra* in both rural and modern households and takes up to 2 hours to cook. Due to its bitter taste the cooking process may involve discarding some of the water prior to adding other ingredients. Some studies report the detrimental effects of boiling on flavonoid content [40], [41], [42]. But in this study the boiling improved both the TPC and TFC of *C. gynandra*. Boiling may encourage the release of dietary fibre and protein bound flavonoids and thereby increasing the amount of free flavonoids in the vegetable material. According to [43] cell walls and cell membranes in plants begin to breakdown when heated above 60°C. In addition, an appreciable amount of polyphenols are found esterified with cell wall carbohydrates [44]. In linking these two statements preceding above it is clear that there could be a release of phenolic compounds from the cell walls that would result in an increase in TPC and TFC during boiling. The boiled sample showed the lowest percentage increase of TPC and TFC most likely due to the leaching of soluble phenolic compounds into cooking water. This is apparent since the boiled sample filtrate showed a considerable amount of TPC and TFC. Steaming has been considered as the most viable method to use during cooking as it preserves higher amounts of nutrients such as vitamin C in vegetables. In this study it also seems to be the method with the highest TPC as compared to the raw vegetable. These results are similar to those studied by [30], where an increase in the range of 18–86% occurred in 8 of the indigenous vegetables studied. Steaming also showed an improvement of flavonoids by 424% as compared to the raw sample. Most studies have reported an increase in flavonoid content after steaming [30], [45], and [46]. The dried sample depicted an increase in both TPC and TFC. According to [24], a slight increase in TFC after drying irish seaweed (*Himanthalia elongate*) for 12 hours was also observed. Even though there was an increase in TPC and TFC after drying, the magnitude is significantly less than other processing methods analysed perhaps due to the activity of plant enzymes that were still active in the process of drying as well as oxidation of phenolic compounds during the drying process since the vegetable was exposed to atmospheric oxygen. Plant enzymes such as polyphenol oxidases and peroxidases are responsible for the oxidation of phenolic compounds such as flavonoids [47]. To prevent such an incident blanching is used as a method to inactivate these plant enzymes. In this experiment blanching improved the TPC and TFC of the raw vegetable significantly. This is consistent with the results of [48] who recorded an increase of 200% and 100% after blanching *Structium sparejanophora* and *Solanum macrocarpon* respectively.

B. Antioxidant Activity

The antioxidant activity of *C. gynandra* was determined by the DPPH, ABTS and FRAP assays and mean values are shown in table 2. There was a significant difference between the processing methods used and the raw sample at $p < 0.05$ for all the antioxidant assays. The steamed sample displayed the highest DPPH, FRAP and ABTS mean values of 499.38 ± 2.44, 578.68 ± 5.19, and 214.39 ± 12.33 µM TE/g respectively. Across all assays used the boiled sample filtrate depicted the lowest activity at 32.50 ± 0.31 µM TE/g for DPPH, 38.88 ± 0.033 µM TE/g for FRAP, and 11.09 ± 0.94 µM TE/g for ABTS. The boiled sample exhibited a decrease in DPPH and FRAP activity at 4.34 % and 0.58 % respectively in comparison to the raw sample. The ABTS and DPPH results of the raw sample are comparable to that of [14] who reported 155. 87 µM TEAC/g and 81.109 µM QEAC/g for ABTS and DPPH respectively. The FRAP assay recorded the highest antioxidant activity as compared to the DPPH and ABTS assay probably showing that *C. gynandra* has a high reducing power which according to [49] reducing power is related to how the phenols are conjugated as well as the number of hydroxyl groups present. In regards to Cooking and drying, the antioxidant activity of raw *C. gynandra* leaves across all assays improved generally with slight decreases occurring with the boiled sample for the DPPH and FRAP assays. A similar trend of a decrease in antioxidant activity after boiling was reported by [41] for spinach and also by [50] for drumstick (*Moringa oleifera*) and pumpkin (*Cucurbita maxima*) leaves. Decreases occurring could have been induced by the leaching of antioxidant compounds into the cooking water. The possibility of such an incident is verified by boiled sample filtrate which had a significant antioxidant activity across all assays. The steamed sample exhibited the highest antioxidant activity across all assays used in analysis. This phenomenon is consistent with the results of [41], [30], [51], [52] who found an increase in antioxidant activity after steaming of green leafy vegetables. The process of steaming would basically increase the extractability of antioxidants from cell wall structures and cell vacuoles without leaching of the compounds into the cooking water. The blanched sample showed a significantly higher

antioxidant activity than the raw sample. Similarly reference [50], reported an increase in DPPH and FRAP activity for drumstick leaves and amaranth leaves respectively. Blanching is a short time high temperature treatment which could possibly open up the cell matrix and induce breakage of covalent bonds in conjugated phenolic compound and hence releasing free phenolic compounds that exhibit a higher antioxidant activity due to the availability of hydroxyl groups. Drying of the leaves at 50°C showed a significantly higher antioxidant activity than the raw sample perhaps due to oxidation of some phenolic compounds that could yield higher antioxidant activity. According to [53], reaction products of the parent antioxidant can still contain residual antioxidant activity. In this study it has been noted that drying of *C. gynandra* is best accompanied by blanching as evidenced by the blanched and dried sample that exhibited a higher antioxidant activity than the dried sample.

TABLE II
EFFECT OF COOKING AND DRYING ON THE RADICAL SCAVENGING ACTIVITY OF *C. GYNANDRA*

Sample Type	DPPH (µM TE/g) dw*	FRAP (µM TE/g) dw*	ABTS (µM TE/g) dw*
Raw	221.21 ± 0.31^{c}	247.58 ± 1.43^{b}	89.91 ± 7.73^{b}
Boiled	211.61 ± 1.97^{b}	246.14 ± 3.59^{b}	102.29 ± 5.26^{bc}
Boiled sample filtrate	32.50 ± 0.31^{a}	38.88 ± 0.033^{a}	11.09 ± 0.94^{a}
Blanched	445.42 ± 6.34^{f}	510.60 ± 9.28^{e}	168.75 ± 19.98^{e}
Dried	253.68 ± 3.18^{d}	288.77 ± 3.35^{c}	107.61 ± 11.37^{c}
Blanched and Dried	310.70 ± 11.18^{e}	347.75 ± 5.62^{d}	129.49 ± 7.17^{d}
Steamed	499.38 ± 2.44^{g}	578.68 ± 5.19^{f}	214.39 ± 12.33^{f}

*Results expressed in dry matter weight (dw). DPPH - 2,2-diphenly-1- picrylhydrazyl assay, FRAP - Ferric Reducing Antioxidant assay, ABTS - 2,2- azinobis (3-ethyl-benzothiazoline-6-sulfonic acid), TE- Trolox Equivalent. All data reported as means ± SD (n=6). Alphabets in superscripts within a column show significant differences among the cooking and drying methods.

C. Correlation between Total phenolic, Flavonoid Content and Radical Scavenging Activity.

An analysis using Pearson's correlation coefficient indicated that there is a significant positive linear relationship between Total phenolic, Total Flavonoid contents and DPPH, FRAP and ABTS assays at $p < 0.01$. The essence of studying the correlation between TPC, TFC and antioxidant activity was to insinuate that the phenolic compounds in *C. gynandra* are highly responsible for its antioxidant activity. A number studies have reported positive correlations between phenolic compounds and antioxidant activity [54], [55], [56], and [57].

D. Antibacterial Activity

Minimum inhibitory concentration (MIC) was used to determine the antimicrobial activity of raw, cooked and dried samples. The dried sample showed the most inhibition activity against 3 bacterial strains i.e. *E. aerogenes, E. coli,* and *P. aeruginosa*. The raw sample only inhibited the activity of *E. aerogenes* at 32 mg/mL while the blanched and dried sample only inhibited the activity of *E. cloacae* also at 32 mg/mL. No inhibition occurred against all bacterial strains for the boiled and blanched samples at the range of 0 - 32 mg/mL concentration of the crude extract. The results of this study illustrate that cooking and drying have an effect on the antibacterial activity of *C. gynandra*. The dried sample depicted the most activity against the three of the four gram negative bacteria. During hot air drying phenolic compounds could have been oxidised and condensed to form more complex compounds with higher antibacterial activity. According to [58] a fusion of phenolic compounds can provide a combined effect on antimicrobial activity which can lead to a greater antimicrobial activity than a single compound. It was also reported that phenolic compounds lacking free hydroxyl groups have a better antibacterial activity than their aglycones due to better hydrophobicity to the microbial lipid membrane [59]. Thus this could explain why there was less or no antibacterial activity recorded for other cooking methods (boiled, steamed, blanched and blanched & dried) which might have more compounds with free hydroxyl groups. *E. aerogenes* seems to be inhibited by the raw and dried samples only probably due to the presence of phenolic compounds with hydrophobic characteristic that were not degraded by cooking water as compared to the other moist methods cooking used. *E. cloacae* growth was only inhibited by extracts that involved moist cooking (steamed and blanched & dried) which helps extract phenolic compounds from cell wall structures while preventing the leaching of those compounds with antibacterial activity.

E. Fourier transform infrared spectroscopy (FTIR)

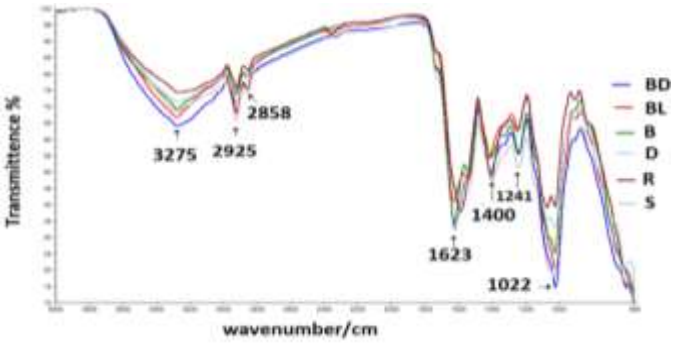

Fig. 1 FTIR spectrum for *C. gynandra* from different processing methods. BD – Blanched & dried sample, BL – Blanched, B - Boiled, D – Dried, R – Raw, S – Steamed

The FTIR spectrum was used to identify phenolic related functional groups occurring in *C. gynandra* . Figure 2 shows FTIR spectrum for *C. gynandra* with different processing conditions. The absorption band at 3278 cm^{-1} could be assigned to the H-bonded hydroxyl group (OH), which mainly appears in the region 3500 – 3200 cm^{-1} [60]. The absorption bands at 2925 cm^{-1} and 2858 cm^{-1} are possibly representing the –CH vibrations of the $–CH_3$ and $–CH_2$ functional groups [61]. The band occurring at 1623 cm^{-1} could probably be due to the presence of conjugated carbonyl bonds from flavonoids or hydroxyl groups [62], [63]. Bands of 1400 cm^{-1} occurring between 1410 cm^{-1} and 1310 cm^{-1} are characteristic of phenols OH functional groups [60]. Generally, bands in the range 1170 – 930 cm^{-1} can be designated to the flavonoids and polysaccharide functional group [62]. The presence of Aromatic compounds can be further assigned to bands occurring at 930 – 700 cm^{-1} due to C-H bending [62]. In general, the peaks identified show the presence of phenolic compounds in all samples. The FTIR spectra of

phenols is mainly characterised by bands affiliated with hydroxyl groups including the bending and stretching vibrations of the O-H and C-O functional groups. The steamed, blanched and the blanched & dried sample exhibited a lower % transmittance in the H- bonded hydroxyl group region as well as the region belonging to the flavonoid or polysaccharide functional group. Lower transmittance relates to the degree and strength of the hydrogen bonding [60] and hence this could explain higher antioxidant activities found in this study.

IV. Conclusion

Given the results sought from this study it is a good indication that cooking and drying imparted positively on the presence of phenolic compounds as well as the antioxidant activity of *C. gynandra* as it is usually cooked prior to consumption. This would imply that the cooking and drying methods used in this study would increase the vegetable's possibility in reducing chronic diseases. Although there was an increase in TPC, TFC of the boiled sample leaching of some phenolic compounds occurs into the cooking water. Hence it is advisable to avoid discarding the cooking water used to cook the vegetable as valuable components could be lost. The antimicrobial properties also indicate that preservation of the vegetable by drying at 50°C will improve the vegetable's antimicrobial properties, therefore it can be used as a functional food ingredient that will assist as an antimicrobial agent. The results obtained from the study as a whole could possibly promote the utilisation of *C. gynandra* as an accompaniment to starchy foods and its use as a value added product in commercial applications as it is still classified as an underutilised plant.

References

[1] G. Pang, J. Xie, Q. Chen, and Z Hu, "How functional foods play critical roles in human health," Food Science and human wellness, vol. 1(1), pp 26-60, December 2012.

[2] I. FAO, "The State of Food Insecurity in the World. Strengthening the enabling environment for food security and nutrition," Food and Agriculture Organization of the United Nations, Rome 2014.

[3] B. Ambrose-Oji. Urban food systems and African indigenous vegetables: Defining the spaces and places for African indigenous vegetables in urban and peri-urban agriculture. African indigenous vegetables in urban agriculture, pp 1-33, September 2009.

[4] A.M. Van der Walt, D.T. Loots, M.I. Ibrahim, C.C Bezuidenhout, "Minerals, trace elements and antioxidant phytochemicals in wild African dark-green leafy vegetables (morogo)," South African Journal of Science, vol. 105(11-12), pp 444-8, December 2009.

[5] M.V. Flyman, A.J. Afolayan, "A survey of plants used as wild vegetables in four districts of Botswana," Ecology of food and nutrition, vol.45 (6), pp 405-415, December 2006.
https://doi.org/10.1080/03670240600985431

[6] R.S. Glew, B. Amoako-Atta, G. Ankar-Brewoo, J. Presley, L.T. Chuang,M. Millson, B.R Smith, and R.H Glew, "Non-cultivated plant foods in West Africa: Nutritional analysis of the leaves of three indigenous leafy vegetables in Ghana," Food, vol 3(1), pp 39-42, 2009.

[7] F.B.O. K'Opondo, "Influence of drying method and fruit position on the mother plant on seed quality of spiderplant (Cleome gynandra L.) morphotypes from western Kenya." Adv. Appl. Sci. Res, vol. 2.3, pp 74-83, 2011.

[8] C. Njeme, N.I. Goduka, G. George, "Indigenous leafy vegetables (imifino, morogo, muhuro) in South Africa: A rich and unexplored source of nutrients and antioxidants," African Journal of Biotechnology, vol. 7(13), May 2014.

[9] M. J. Moshi, D.F. Otieno, and A. Weisheit, "Ethnomedicine of the Kagera Region, north western Tanzania. Part 3: plants used in traditional medicine in Kikuku village, Muleba District," Journal of ethnobiology and ethnomedicine, vol. 8.1, April 2012.

[10] M. Muchuweti, C. Mupure, A. Ndhlala, T. Murenje, M. A Benhura, "Screening of antioxidant and radical scavenging activity of Vigna ungiculata, Bidens pilosa and Cleome gynandra," American Journal of Food Technology, vol. 2(3), pp 161-8, 2007.
https://doi.org/10.3923/ajft.2007.161.168

[11] B. Rastogi, U. Tiwari, A. Dubey, B. Bawra, D. Nandini, N. S Chauhan, and D.K Saraf, "Immunomodulating activity of Cleome gynandra," Pharmacologyonline, vol. 2, pp 151-69, 2009.

[12] T. Shanmuganathan, and A. V. P Karthikeyan, "Free radical scavenging activity of aqueous and ethanolic extracts of wild and l-arginine treated cleome gynandra l," vol. 5(07), pp.346–349, 2014.

[13] N.T. Meda, M.J. Bangou, S. Bakasso, J. Millogo-Rasolodimby, O. G. Nacoulma, "Antioxidant activity of phenolic and flavonoid fractions of Cleome gynandra and Maerua angolensis of Burkina Faso," Journal of Applied Pharmaceutical Science, vol. 3(2), pp 36, 2013.

[14] A.N.Brain, M. U. John, "Phenolic content and antioxidant activity of selected Ugandan traditional medicinal foods," African Journal of Food Science, vol. 8(8), pp 427-34, August 2014.
https://doi.org/10.5897/AJFS2014.1136

[15] Y. Konan, K. M. Witabouna, B. Bassirou, K. Kagoyire, "Antioxidant activity and total phenolic content of nine plants from Côte d'Ivoire (West Africa)," Journal of Applied Pharmaceutical Science, vol. 4(8), pp 36, August 2014.

[16] M. Moyo, S.O. Amoo, B. Ncube, A.R. Ndhlala, J. F. Finnie, J. Van Staden, "Phytochemical and antioxidant properties of unconventional leafy vegetables consumed in southern Africa," South African Journal of Botany, vol. 84, pp 65-71, January 2013.
https://doi.org/10.1016/j.sajb.2012.09.010

[17] N. Sridhar, B.V. Kiran, D.T. Sasidhar, L. K Kanthal, "In vitro antimicrobial screening of methanolic extracts of Cleome chelidonii and Cleome gynandra. Bangladesh Journal of Pharmacology, vol. 9(2), pp 161-166, March 2014.
https://doi.org/10.3329/bjp.v9i2.17759

[18] A. Annadurai, and J.S Ahmed, "Preliminary phytochemical screening in Cleome gynandra L," Advances in Applied Science Research, pp.122-124.

[19] A. Osama, and S. Awdelkarim,"Phytochemical screening of Ficus sycomorus L . bark and Cleome gynandra L . aerial parts," vol. 4(4), pp.24–27, 2015.

[20] A. Bala, P.K Haldar, B. Kar, S. Naskar, P. Saha, S KunduSen, M. Gupta, U.K. Mazumder, "Antioxidant activity of the fractions of Cleome gynandra promotes antitumor activity in Ehrlich Ascites Carcinoma," Asian Journal of Chemistry, vol. 23(11), pp 5055, 2011.

[21] J. C. Kalita," Zoology Phytochemical Screening of the Leaves of Cleome Gynandra Linn (Cleomaceae) Jupitara Deka," vol.(7), pp.291–293. 2014

[22] L.I Sowunmi, A.J. Afolayan, "Phytochemical constituents and antioxidant properties of acetone extract of Cleome gynandra (L.) growing in the Eastern Cape, South Africa," African Journal of Traditional, Complementary and Alternative Medicines, vol. 12(13), 2015.
https://doi.org/10.4314/ajtcam.v12i3.1

[23] B. Chipurura, M. Muchuweti, and A. Kasiyamhuru, A, "Wild leafy vegetables consumed in Buhera District of Zimbabwe and their phenolic compounds content," Ecology of food and nutrition, vol.52 pp.178–89 November 2013, Available at: http://www.ncbi.nlm.nih.gov/pubmed/23445395.
https://doi.org/10.1080/03670244.2012.706094

[24] S. Cox, N. Abu-Ghannam, and S.Gupta, "Effect of processing conditions on phytochemical constituents of edible irish seaweed himanthalia elongata,"

[25] G.L Hostetler, K. M. Riedl, and S. J. Schwartz,"Effects of food formulation and thermal processing on flavones in celery and chamomile". Food Chemistry, vol. 141(2), pp.1406–1411, 2013 Available at: http://dx.doi.org/10.1016/j.foodchem.2013.04.051.
https://doi.org/10.1016/j.foodchem.2013.04.051

[26] S. Nithiyanantham, S. Varadharajan, S. and P. Siddhuraju, "Differential Effects of Processing Methods on Total Phenolic

Content, Antioxidant and Antimicrobial Activities of Three Species of Solanum". , vol. 20(4), pp.844–854, 2012

[27] B. Sultana, and F.Anwar,"Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants," Food Chemistry, vol. 108(3), pp 879-884, 2008. https://doi.org/10.1016/j.foodchem.2007.11.053

[28] H.N. Pham, V. T. Nguyen, Q. V Vuong, M. C Bowyer, C. J Scarlett,"Effect of extraction solvents and drying methods on the physicochemical and antioxidant properties of helicteres hirsuta lour leaves," Technologies, vol. 3(4), pp 285-301, December 2015. https://doi.org/10.3390/technologies3040285

[29] I. Juániz, L. A. Ludwig, E. Huarte, G. Pereira-Caro, J. M. Moreno-Rojas, C. Cid, M. P. De Peña," Influence of heat treatment on antioxidant capacity and (poly) phenolic compounds of selected vegetables," Food chemistry, vol. 197, pp 466-473, April 2016. https://doi.org/10.1016/j.foodchem.2015.10.139

[30] S. A. Adefegha, G. Oboh, "Enhancement of total phenolics and antioxidant properties of some tropical green leafy vegetables by steam cooking," Journal of Food Processing and Preservation, vol. 35(5), pp 615-622, October 2011. https://doi.org/10.1111/j.1745-4549.2010.00509.x

[31] M. Palermo, N. Pellegrini, and V. Fogliano, "The effect of cooking on the phytochemical content of vegetables," Journal of the Science of Food and Agriculture, vol. 94(6), pp 1057-1070, 2014. https://doi.org/10.1002/jsfa.6478

[32] A. Michalak,"Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress," Polish Journal of Environmental Studies, vol. 15(4), pp 523, 2006.

[33] O. Oiv, "Compendium of International Methods of Wine and Must Analysis," International Organisation of Vine and Wine: Paris, pp 154-196, France. 2009.

[34] M. A. Al-Farsi, and C. Y. Lee,"Optimization of phenolics and dietary fibre extraction from date seeds," Food Chemistry, vol. 108(3), pp 977-985, 2008. https://doi.org/10.1016/j.foodchem.2007.12.009

[35] F. Sabli, M. Mohamed, A. Rahmat, A. Bakar, and M. Fadzelly, "Cytotoxic properties of selected Etlingera spp. and Zingiber spp.(Zingiberaceae) endemic to Borneo. Pertanika," Journal of Tropical Agricultural Science, vol. 35(3), pp 663-671, 2012.

[36] Y.J. Lee, D. B. Kim, J. S. Lee, J. H. Cho, B. K. Kim, H.S. Choi, and O.H. Lee,"Antioxidant activity and anti-adipogenic effects of wild herbs mainly cultivated in Korea," Molecules, vol.18(10), pp 12937-12950, 2013. https://doi.org/10.3390/molecules181012937

[37] A. A. Adedapo, F. O. Jimoh, A. J. Afolayan, and P. J. Masika, "Antioxidant properties of the methanol extracts of the leaves and stems of Celtis Africana," Rec Nat Prod, vol. 3(1), pp 23-31, 2009.

[38] J. M. Andrews,"Determination of minimum inhibitory concentrations," Journal of antimicrobial Chemotherapy, vol. 48(1), pp 5-16, 2001. https://doi.org/10.1093/jac/48.suppl_1.5

[39] T. Anbazhagi, K. Kadavul, G. Suguna, and A. J. A. Petrus,"Studies on the pharmacognostical and in vitro antioxidant potential of Cleome gynandra Linn. Leaves," Nat. Prod. Rad, vol. 8(2), pp 151-157, 2009.

[40] A. F. Vinha, R. C. Alves, S. V. Barreira,A .S. Costa, and M. B. P. Oliveira,"Impact of boiling on phytochemicals and antioxidant activity of green vegetables consumed in the Mediterranean diet," Food & function, vol. 6(4), 1157-1163, 2015. https://doi.org/10.1039/C4FO01209G

[41] T. Mazzeo, D. N'Dri, E. Chiavaro, A. Visconti, V. Fogliano, and N. Pellegrini,"Effect of two cooking procedures on phytochemical compounds, total antioxidant capacity and colour of selected frozen vegetables," Food Chemistry, vol.128(3), pp 627-633, 2011. https://doi.org/10.1016/j.foodchem.2011.03.070

[42] F. A. Ahmed, and R.F. Ali,"Bioactive compounds and antioxidant activity of fresh and processed white cauliflower," BioMed research international, 2013. https://doi.org/10.1155/2013/367819

[43] J. J. Provost, K. L. Colabroy, B. S. Kelly, M. A. Wallert, "The Science of Cooking" Understanding the Biology and Chemistry behind Food and Cooking, 2016, John Wiley & Sons, Inc Hobeken, New York Jersey. pp 245. https://books.google.co.za/books?id=o-0XDAAAQBAJ.

[44] B. Harbaum, E. M. Hubbermann, C. Wolff, R. Herges, Z. Zhu, and K. Schwarz,"Identification of Flavonoids and Hydroxycinnamic Acids in Pak Choi Varieties (Brassica campestris L. ssp. chinensis var. communis) by HPLC–ESI-MS n and NMR and Their Quantification by HPLC–DAD," J. of Agricultural and Food Chemistry, vol. 55(20), pp 8251-8260, 2007. https://doi.org/10.1021/jf071314+

[45] S. Jin, C. Chang, L Zhang, Y. Liu, X. Huang and Z. Chen, Z, "Chlorogenic acid improves late diabetes through adiponectin receptor signaling pathways in db/db mice," PloS one, vol.10(4), 2015. https://doi.org/10.1371/journal.pone.0120842

[46] N. Do Thi, and E. S. Hwang,"Effects of different cooking methods on bioactive compound content and antioxidant activity of water spinach (Ipomoea aquatica)," Food Science and Biotechnology, vol. 24(3), pp 799-806, 2015. https://doi.org/10.1007/s10068-015-0104-1

[47] B. Nayak, R. H Liu, and J. Tang,"Effect of Processing on Phenolic Antioxidants of Fruits, Vegetables, and Grains—A Review," Critical reviews in food science and nutrition, vol. 55(7), pp 887-918, 2015. https://doi.org/10.1080/10408398.2011.654142

[48] G. Oboh ,"Effect of blanching on the antioxidant properties of some tropical green leafy vegetables," LWT-Food Science and Technology, vol. 38(5), pp 513-517, 2005. https://doi.org/10.1016/j.lwt.2004.07.007

[49] B. D. Craft, A. L. Kerrihard, R. Amarowicz, and R. B. Pegg,"Phenol-based antioxidants and the in vitro methods used for their assessment," Comprehensive Reviews in Food Science and Food Safety, vol. 11(2), pp 148-173, 2012. https://doi.org/10.1111/j.1541-4337.2011.00173.x

[50] C. N. Kunyanga, J. K. Imungi, M. W. Okoth, H. K. Biesalski, and V. Vadivel, "Total phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed Kenyan indigenous food ingredients," LWT-Food Science and Technology, vol. 45(2), pp 269-276, 2012. https://doi.org/10.1016/j.lwt.2011.08.006

[51] M. Şengül, H. Yildiz, and A Kavaz," The effect of cooking on total polyphenolic content and antioxidant activity of selected vegetables," International Journal of Food Properties, vol. 17(3), pp 481-490, 2014. https://doi.org/10.1080/10942912.2011.619292

[52] C. Miglio, E. Chiavaro, A. Visconti, V. Fogliano, and N. Pellegrini," Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables," Journal of agricultural and food chemistry, vol. 56(1), pp 139-147, 2007. https://doi.org/10.1021/jf072304b

[53] G. R. Haenen, M.J. Arts, A. Bast, and M. D. Coleman, "Structure and activity in assessing antioxidant activity in vitro and in vivo: A critical appraisal illustrated with the flavonoids," Environmental toxicology and pharmacology, vol. 21(2), pp 191-198, 2006. https://doi.org/10.1016/j.etap.2005.07.010

[54] T. N. Wen, K. N. Prasad, B. Yang, and A. Ismail, "Bioactive substance contents and antioxidant capacity of raw and blanched vegetables," Innovative Food Science & Emerging Technologies, vol. 11(3), pp 464-469, 2010. https://doi.org/10.1016/j.ifset.2010.02.001

[55] G. R. Genwali, P. P. Acharya, and M. Rajbhandari, "Isolation of Gallic Acid and Estimation of Total Phenolic Content in Some Medicinal Plants and Their Antioxidant Activity," N https://doi.org/10.3126/njst.v14i1.8928

[56] V. Pakade, E. Cukrowska, and L. Chimuka, "Comparison of antioxidant activity of Moringa oleifera and selected vegetables in South Africa," South African Journal of Science, vol. 109(3-4), 2013. https://doi.org/10.1590/sajs.2013/1154

[57] R. D. Katerere, "Antioxidant activity of some African medicinal and dietary leafy African vegetables," African Journal of Biotechnology, vol. 11, 2012.

[58] S. A. Hayek, R. Gyawali, and S. A Ibrahim, "Antimicrobial natural products. Microbial pathogens and strategies for combating them," Science, technology and education, vol. 2, pp 910-921, 2013.

[59] L. W. Nitiema, A. Savadogo, J. Simpore, D. Dianou, and A. S. Traore, "In vitro antimicrobial activity of some phenolic compounds (coumarin and quercetin) against gastroenteritis bacterial strains," International Journal of Microbiological Research, vol. 3(3), pp 183-187, 2012.
[60] J. Coates, "Interpretation of infrared spectra, a practical approach," Encyclopedia of analytical chemistry, 2000.
[61] A. M. Awwad, N. M. Salem, and A. O Abdeen, "Biosynthesis of Silver Nanoparticles using Olea europaea Leaves Extract and its Antibacterial Activity," vol. 2(1), pp.164–170, 2012.
[62] A. D. Buzoianu, C. Socaciu. "Untargeted Metabolomics for Sea Buckthorn (Hippophae rhamnoides ssp. carpatica) Berries and Leaves: Fourier Transform Infrared Spectroscopy as a Rapid Approach for Evaluation and Discrimination," Notulae Botanicae Horti Agrobotanici Cluj-Napoca. Vol, 42(2), pp 545-50, December 2014.
[63] R. M. Pop, "Metabolomic studies applied on different seabuckthorn (Hippophae Rhamnoides L.) varieties," University of Agricultural Science and Veterinary Medicine Cluj Napoca, PhD. Diss, 2012.

S. M Moyo is a student currently doing her masters in Food Technology at the University of Johannesburg, South Africa. She was born in the city of Bulawayo, Zimbabwe on the 9th of June 1988. She graduated from the University of Namibia, Windhoek, Namibia in April 2013 with an honours degree in Food Science and Technology. During her undergraduate studies, she interned at a meat processing company (Colcom Foods) in Harare, Zimbabwe as a quality controller. She also interned at the Central Veterinary Laboratory (CVL) Namibia as a laboratory technician in the food hygiene, biotechnology and toxicology residue analysis departments. After receiving her honours degree, she worked as a teacher at Mtshabezi high school Zimbabwe and taught food science and food and nutrition subjects. She is currently Lecturing Food Technology at the University of Johannesburg