Antioxidant Activity by DPPH Assay of Crude Methanolic Extracts from Selected Indigenous Vegetables in Kenya

Palmer Sivoko Imbenzi[1]*, Lily Ateko Masinde[1], Joel Obuya[1]

[1]Kenya Methodist University P.O. Box 627 – 60200, Meru, Kenya.

**Correspondence email: palmer.imbenzi@kemu.ac.ke*

Abstract

Antioxidants are natural or synthetic biological molecules that prevent cell damage by averting free radical formation, scavenging, or promoting their decomposition. When the body's ability to counteract free radicals is overburdened, oxidative stress develops, triggering several diseases. Indigenous vegetables are beneficial sources of nutrition that can supply the recommended daily allowance of nutrients with antioxidants. In this study, five indigenous vegetables (*Cleome gynandra, Solanum nigrum, Basella Alba, Cucurbita argyrosperma,* and *Amaranthus blitum*) collected from local markets in Kenya were investigated for the presence of antioxidants using the two assays: 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and Thin Layer Chromatography (TLC). 100 µg/ml of the crude extract was spotted on the Alugram Xtra GUV254 TLC plate. The chromatogram was developed using methanol (95% volume): ethylacetate (5% volume). The dried chromatogram was sprayed with DPPH (0.15 % w/v) solution and examined after 30 minutes. The positive control of this assay was vitamin C. Total flavonoid and phenol contents were used to validate the antioxidants present. A stable DPPH radical is purple in color, on reduction it changed to yellow diphenyl picryl hydrazine compound, indicating active free-radical scavenging compounds present in the crude extracts. The intensity of the yellow color denoted their high scavenging ability. The antioxidants, phenolic and flavonoid contents had a good correlation in a dose-dependent manner. These results indicated indigenous vegetables contain antioxidant compounds. This study recommends investigation using *In-vitro* antioxidant assays on each of the crude extracts to determine their scavenging capacities and spectroscopy to determine the structure of the active antioxidant compounds. The study equally recommends a comparative study on indigenous vegetables from different ecological zones to determine the geographical influence of the availability of antioxidants. Promoting the use of indigenous vegetables and maximizing their potential for food can help accomplish some objectives of Vision 2030 and the Sustainable Development Goals.

Keywords: *Indigenous vegetables, Antioxidants, Flavonoids, Phenols, SDGs*

IJPP 11(3); 16-25

1.0 Introduction

According to Halliwell (1994), free radicals are atoms or molecules that possess one or more unpaired electrons that cause them to become highly reactive. Primary radicals formed and encountered by cells include hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet-}$), and nitric oxide ($NO^{\bullet-}$) (Freeman & Crapo, 1982). Plasma membrane or intracellular enzymes and proteins are cellular sources of free. Some exogenous sources of free radicals from lifestyle and environment are radiation, air pollutants, tobacco, and drinking alcohol (Freeman & Crapo, 1982).

When left unchecked, free radicals can damage human deoxyribonucleic acid (DNA) by robbing other biomolecules of their electrons through a process known as oxidation (Halliwell, 1994; Rackova et al., 2007). The existence of radical-generating agents or reactive oxygen species (ROS) in concentrations that overpower the body's natural mechanisms for blocking or scavenging radicals is what triggers oxidative stress (OS) (Nunomura, 2013; Padurariu et al., 2013; Shruthi et al., 2012). These can damage DNA, lipids and proteins, which has been linked to several clinical conditions, including autoimmune disorders, cancer, cataracts, cardiovascular disease, aging, rheumatoid arthritis, and neurodegenerative diseases (Pham-Huy et al., 2008; Reynolds et al., 2007).

Plant-based foods are known for their high antioxidant content which function as chemopreventive agents against cell damage (Shruthi et al., 2012; Shu-Jing & Lean-Teik, 2008), thereby preventing diseases like coronary heart disease and atherosclerosis as well as longevity. African communities have traditionally grown and utilized traditional vegetables but the potential of these vegetables has been inadequately exploited (Abukutsa, 2007; Schippers, 2000). Studies have shown that the popular exotic vegetables like cabbage, broccoli, kale, cauliflower, brussel sprouts are lower in mineral and vitamin contents than indigenous vegetables. On average 100g of fresh indigenous vegetable contain levels of calcium, iron, phenolics and vitamins that would provide 100% of the daily requirement (Abukutsa, 2003). Therefore indigenous vegetables are important in complementing the body's defence mechanism against free radicals (Schippers, 2000).

"Plant-based foods are known for their high antioxidant content which function as chemopreventive agents against cell damage"

Polyphenols are vital phytonutrients of plant origin that can be incorporated as functional ingredients in foods. Ranging from simple

phenolic molecules to highly polymerized compounds such as tannins. Recent interest in food phenolics has increased greatly owing to their antioxidant capacity, that is, free radical scavenging and metal chelating activities, and their possible beneficial implications in human health, such as in the treatment and prevention of cancer, cardiovascular disease, and other pathologies (Shahidi & Ambigaipalan, 2015). Polyphenolics offer an effective natural therapy against obesity via modulating gut microbiota composition (Aloo et al., 2023; Ozdal et al., 2016).

Lignans consist of compounds mainly found in fruits, vegetables, coffee, tea, and cereal products which when consumed express anti-obesity activity by inhibiting the expression of adipogenic factors and lipid metabolism-regulating factors during adipocyte differentiation. They are also said to be involved in inducing G0/G1 cell cycle arrest; hence, inhibiting mitotic clonal expansion during the early stage of adipogenesis (Aloo et al., 2023; Lee et al., 2020).Indeed, promoting the use of indigenous vegetables and maximizing their potential for food can help accomplish some objectives of vision 2030 and the Sustainable Development Goals (SDGs). Commonly used indigenous vegetables in Kenya are amaranths (*Amaranthus* species), African nightshade (*Solanum* species), African kale (*Brassica carinata*), spider plant (*Cleome gynandra*), cowpeas (*Vigna unguiculata*), African eggplant (*Solanum aethiopicum*), jute mallow (*Corchorus olitorius*), and several others (Abukutsa, 2007)

2.0Materials and methods

Collection and preparation of vegetable parts for extraction

The selected indigenous vegetables (Table 1) were purchased from local markets in Kenya (Meru and Kisii). The taxonomic identity of the study plants was confirmed by a botanist at the East African Herbarium (EA), and National Museums of Kenya, Nairobi. The vegetable parts were cleaned using tap water and dried using a drier and under shade to a constant weight. After drying, the samples were ground into a fine powder using a blender (MBLR402/BLX model), packed, labelled, and stored at room temperature in preparation for extraction.

Table 1

Indigenous vegetable samples

Scientific name	Location of collection	Common name	Local name	Edible parts
Cleome gynandra	Kisii	Spider plant	Chinsaga	Leaves & stems
Solanum nigrum	Kisii	Nightshade	Amanagu/Rinagu	Leaves & stems
Basella alba	Kisii	Malabar spinach	Nderema	Leaves
Cucurbita argyrosperma	Meru	Pumpkin leaves	Marenge	Leaves
Amaranthus blitum	Meru	Amaranths	Terere	Leaves & stems

Preparation of crude extracts from the pulverized vegetable parts

The ground powder of each vegetable sample was soaked separately in methanol and then covered with aluminium foil. Extraction was allowed to proceed for 72 hours while shaking the contents after every 12 hours. The crude extracts were obtained by decanting, filtering and concentrating under reduced pressure to remove the solvent. The crude extracts are then air dried, labelled, and stored in a sterile beaker at room temperature (Emynur Shafekh et al., 2012; Panichayupakaranant et al., 2010).

Antiradical screening by thin layer chromatography (TLC)

To assess the antioxidant activity of each raw extract, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay was conducted. The methanol extract of 1 mg/mL was applied on a TLC plate of Alugram Xtra GUV254 as spot at a concentration of 100 µg/mL. The mobile phase used for the separation was a mixture of methanol and ethylacetate in a ratio of 95:5 (v/v). The developed chromatogram was dried, and a DPPH solution in methanol (0.15% w/v) was sprayed using an atomizer over the entire plate. After 30 minutes, the plate was examined. The positive control of this assay was vitamin C. The colour of a stable DPPH radical is purple, but on reduction, it should give yellow-coloured diphenyl picryl hydrazine compound (Kumar et al., 2014).

Total flavonoid content

The total quantity of flavonoids present was determined following the protocol used by (Imbenzi et al., 2014; Zou et al., 2004), in which 0.5 mL of crude extract solution was mixed with 2 mL of distilled water. Then, 0.15 mL of a 5% $NaNO_2$ solution was added, and the mixture was incubated for 6 minutes. Next, 0.15 mL of a 10% $AlCl_3$ solution was added and allowed to stand for another 6 minutes. After that, the solution was mixed with 2 mL of a 4% NaOH solution and diluted with distilled water to a final volume of 5 mL with thorough mixing. After 15 minutes of standing, the absorbance was measured at 510 nm wavelength. The quantity of flavonoids was expressed as mg of gallic acid equivalents (GAE) per gram of the bioactive crude extract.

Total phenol content

To determine the total phenol content of each methanolic extract, the Folin–Ciocalteu method was used. The method involved mixing 2 g of the sample with 2.5 mL of distilled water, 0.5 mL of Folin–Ciocalteu stock reagent, and 1.0 mL of Na_2SO_4 reagent (75 g/l). The mixture was then incubated at room temperature for 30 minutes before measuring the absorbance at 765 nm wavelength. The total phenolic content was calculated and expressed as mg of gallic acid equivalents per 100 mg of the bioactive crude extract (Cliffe et al., 1994; Imbenzi et al., 2014).

3.0 Results and Discussion

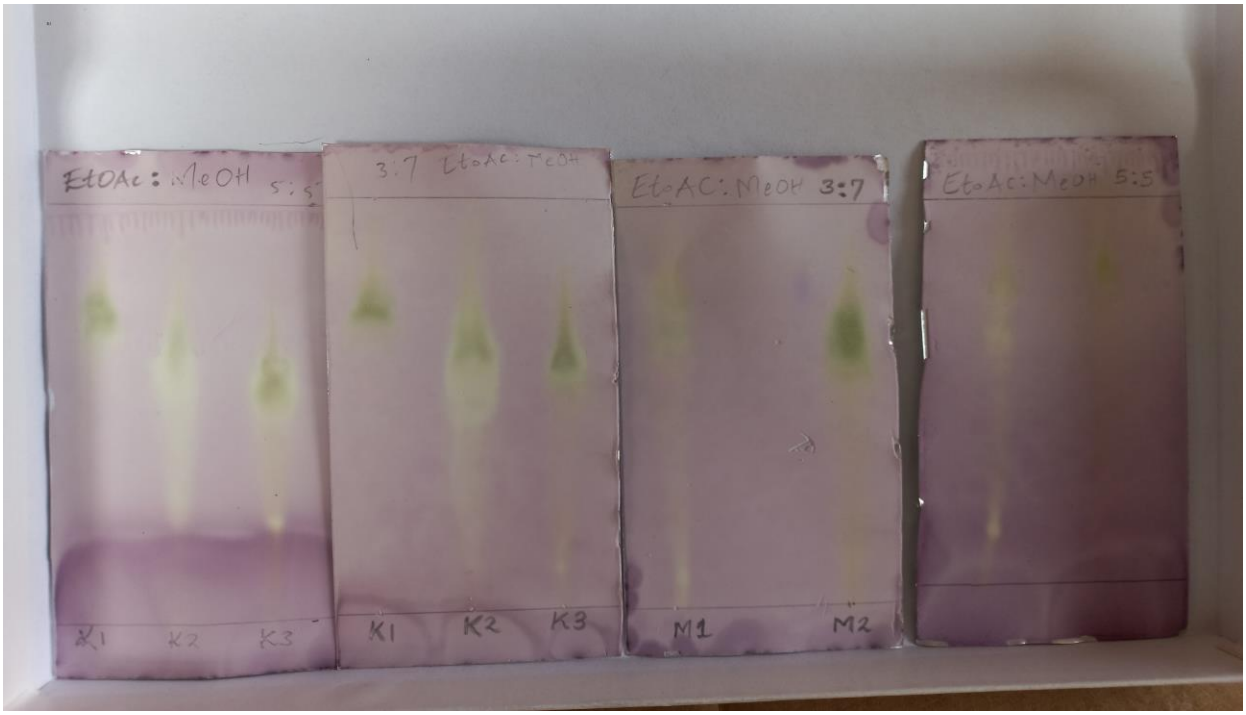
Free-radical scavenging activity

The use of the thin layer chromatography (TLC) method for antiradical screening allowed for a quick, efficient and straightforward analysis of the plant extract

profiles from selected indigenous vegetables. The technique enabled simultaneous separation and measurement of radical scavenging activity of antioxidative compounds without requiring sample purification. This approach allowed for both qualitative and semi-quantitative analysis of antioxidants. The appearance of yellow colour on the purple background of the TLC plate was considered an indication of the presence of antioxidant substances (Figure 1).

Figure 1

TLC plates showing variations of spots colour change on spraying with DPPH (K1: Cleome gynandra, K2: Solanum nigrum, K3: Basella alba, M1: Cucurbita argyrosperma, M2: Amaranthus blitum)



Total flavonoid content

Gallic acid equivalents were used to quantify the amount of flavonoids present in the extracts. Both extracts exhibited a considerable amount of flavonoid content (Table 2).

Table 2

Total flavonoid content of Cleome gynandra, Solanum nigrum, Basella alba Cucurbita argyrosperma, Amaranthus blitum and gallic acid

Concentration (mg/mL)	Total flavonoid content (mg/g) GAE					
	C. gynandra	***S. nigrum***	***B. alba***	***C. argyrosperma***	***A. blitum***	**GAE**
0.1	1.57	1.21	1.18	1.16	1.07	0.63
0.2	1.60	1.58	1.42	1.38	1.26	1.18
0.3	2.00	1.89	1.86	1.78	1.70	2.59
0.4	2.29	2.21	2.15	2.09	2.03	3.18
0.5	2.58	2.48	2.37	2.28	2.13	4.15

A greater concentration of extract was found to be associated with a higher flavonoid content. At a concentration of 0.1 mg/mL, all extracts exhibited the lowest flavonoid content. Among the extracts, the methanolic extract of *Cleome gynandra* exhibited the highest flavonoid content at 1.57 mg/g, while that of *Amaranthus blitum* had the lowest at 1.07 mg/g. As the extract Concentration increased, the flavonoid content also increased.

Total phenol content

The Folin Ciocalteu reagent was used to determine the total phenolic content of the extracts expressed as gallic acid equivalents. The methanolic extracts demonstrated a dose-dependent increase in phenolic content and displayed relatively high levels of phenols (Table 3).

Table 3

Total phenol content of Cleome gynandra, Solanum nigrum, Basella alba Cucurbita argyrosperma, Amaranthus blitum and gallic acid

Concentration (mg/mL)	Total phenol content (mg/g) GAE					
	C. gynandra	***S. nigrum***	***B. alba***	***C. argyrosperma***	***A. blitum***	**GAE**
0.1	1.47	1.15	1.11	1.03	0.93	0.58
0.2	1.58	1.32	1.28	1.11	1.07	1.15
0.3	1.87	1.49	1.33	1.26	1.18	2.43
0.4	2.04	1.83	1.75	1.69	1.37	3.09
0.5	2.18	2.08	1.97	1.88	1.73	4.03

A colorimetric assay is used to detect phenolic and polyphenolic antioxidants by utilizing the Folin-Ciocalteu reagent, which is a combination of phosphomolybdate and

phosphotungstate. The reagent's oxidation is inhibited by the substance being tested, and the amount of substance required for inhibition is measured. Additionally, the color changes after storage may indicate the presence of phenols in samples, since plant extracts' antioxidant properties act as a reducing agent in redox reactions.

The concentration of the extract was directly proportional to the total phenolic content, as expressed in gallic acid equivalents (GAE). At a concentration of 0.1 mg/mL, the phenolic content of all extracts was at its lowest. The *Cleome gynandra* methanolic extract exhibited the highest phenolic content (1.47 mg/g), while the *Amaranthus blitum* methanolic extract had the lowest phenolic content (0.93 mg/g).

4.0 Conclusion

The study concluded that *Cleome gynandra* is high in phenolic compounds, and presence of antioxidants in methanolic extract as compared to *Cucurbita argyrosperma, Solanum nigrum, Amaranthus blitum,* and *Basella alba*. The antioxidants, phenolics and flavonoids present had a correlation with the concentration of the extracts. Methanolic extracts of all the five indigenous vegetables showed comparatively good phenolic content in a dose dependent manner. These results indicate indigenous vegetables contain potential natural compounds with antioxidants that can minimise cell damage.

5.0 Recommendations

In-vitro antioxidant assays is recommended on each of the selected indigenous vegetables' crude extracts to determine their scavenging capacities. The crude extracts should be subjected to isolation, purification and structure elucidation to determine structure of the active antioxidant compounds present. Other techniques of sample drying like oven drying, direct sun drying and methods of cooking the indigenous vegetables can be used to investigate their effect on the quantity of antioxidants in the final product. Comparative study should be done on indigenous vegetables from different ecological zone to determine how geographical factors may influence the availability of antioxidant compounds.

Acknowledgement

The authors would like to acknowledge Kenya Methodist University through the Directorate of Research, Innovations and Extension for providing the research grants under the Internally Funded Research Projects - KeMU 1st call 2022 and laboratory infrastructure for conducting the research.

References

Abukutsa, M. O. O. (2003). Unexploited potential of Indigenous African Vegetables in Western Kenya. *Maseno Journal of Education Arts and Science*, *4*(1), 103-122. https://www.maseno.ac.ke/maseno-journals

Abukutsa, M. O. O. (2007). The diversity of cultivated African leafy vegetables in three communities in western Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, *7*(3), 1-15. https://doi.org/10.18697/ajfand.14.IPGRI1-3

Aloo, S.-O., Ofosu, F. K., Kim, N.-H., Kilonzi, S. M., & Oh, D.-H. (2023). Insights on Dietary Polyphenols as Agents against Metabolic Disorders: Obesity as a Target Disease. *Antioxidants*, *12*(2), 416. https://doi.org/10.3390/antiox12020416

Cliffe, S., Fawar, M. S., Maier, G., Takata, K., & Ritter, G. (1994). Enzyme assays for the phenolic content of natural juices. *Journal of Agricultural and Food Chemistry*, *42*, 1824-1828. https://doi.org/10.1021/jf00044a048

Emynur Shafekh, S., Mohd Adzim Khalili, R., Catherine, C. C. W., Siti Syakiroh, Z. A., Ummu Habibah, A., Norhayati, A. H., . . . & Ahmad Zubaidi, A. L. (2012). Total phenolic content and *in vitro* antioxidant activity of *Vigna sinensis*. *International Food Research Journal*, *19*(4), 1393-1400. http://agris.upm.edu.my:8080/dspace/handle/0/11756

Freeman, B. A., & Crapo, J. D. (1982). Biology of disease: free radicals and tissue injury. *Laboratory Investigation*, *47*(5), 12-26. https://pubmed.ncbi.nlm.nih.gov/7287745/

Halliwell, B. (1994). Free radicals and antioxidants: a personal view. *Nutrition reviews*, *52*, 253-265. https://doi.org/10.1111/j.1753-4887.1994.tb01453.x

Imbenzi, P. S., He, Y.-z., Yan, Z.-x., Osoro, E. K., & Cheplogoi, P. K. (2014). Chemical constituents in extracts from leaves of Lantana trifolia and their in vitro antioxidative activity. *Chinese Herbal Medicines*, *6*(3), 242-246. https://doi.org/10.1016/S1674-6384(14)60035-6

Kumar, S., Sandhir, R., & Ojha, S. (2014). Evaluation of antioxidant activity and total phenol in different varieties of Lantana camara leaves. *BMC Research Notes*, *7*(1), 1-9. https://doi.org/10.1186/1756-0500-7-560

Lee, H., Jeong, J. H., & Ryu, J.-H. (2020). Lignan from Alnus japonica inhibits adipocyte differentiation via cell cycle and FOXO1 regulation. *Molecules*, *25*(15), 3346. https://doi.org/10.3390/molecules25153346

Nunomura, A. (2013). Oxidative stress hypothesis for Alzheimer's disease and its potential therapeutic implications. *Rinsho Shinkeigaku, 53*, 1043-1045. https://doi.org/10.5692/clinicalneurol.53.1043

Ozdal, T., Sela, D. A., Xiao, J., Boyacioglu, D., Chen, F., & Capanoglu, E. (2016). The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. *Nutrients*, *8*(2), 78. https://doi.org/10.3390/nu8020078

Padurariu, M., Ciobica, A., Lefter, R., Serban, I. L., Stefanescu, C., & Chirita, R. (2013). The oxidative stress hypothesis in Alzheimer's disease. *Psychiatria Danubina*, *25*, 401-409. https://hrcak.srce.hr/162117

Panichayupakaranant, P., Tewtrakul, S., & Yuenyongsawad, S. (2010). Antibacterial, anti-inflammatory and anti-allergic activities of standardized pomegranate rind extract *Food Chemistry*, *123*, 400–403. https://doi.org/10.1016/j.foodchem.2010.04.054

Pham-Huy, L. A., He, H., & Pham-Huyc, C. (2008). Free Radicals, Antioxidants in Disease and Health. *International Journal of Biomedical Science*, *4*(2), 89-96. https://pubmed.ncbi.nlm.nih.gov/23675073/

Rackova, L., Oblozinsky, M., Kostalova, D., Kettmann, V., & Bezakova, L. (2007). Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. *Journal of Inflammation*, *4*, 15. https://doi.org/10.1186/1476-9255-4-15

Reynolds, A., Laurie, C., Mosley, R. L., & Gendelman, H. E. (2007). Oxidative stress and the pathogenesis of neurodegenerative disorders. *International Review of Neurobiology*, *82*, 297-325. https://doi.org/10.1016/S0074-7742(07)82016-2

Schippers, R. R. (2000). African indigenous vegetables. An overview of the cultivated species. In *Horticultural Development Services* (Revised CD-ROM ed., pp. 25-31, 147-192). http://r4d.dfid.gov.uk/Output/179575/

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of functional foods*, *18*, 820-897. https://doi.org/10.1016/j.jff.2015.06.018

Shruthi, S. D., Adhikari, R., & Kumar, H. N. (2012). A Review on Medicinal Importance of *Basella Alba* L. *International Journal of Pharmaceutical Sciences and Drug Research*, *4*(2), 110-114. https://oaji.net/articles/2014/364-1401974410.pdf

Shu-Jing, W., & Lean-Teik, N. (2008). MAPK inhibitors and pifithrin-alpha block cinnamaldehyde-induced

apoptosis in human PLC/PRF/5 cells. *Food and Chemical Toxicology*, *45*(12), 2446-2453. https://doi.org/10.1016/j.lfs.2005.02.005

Zou, Y., Lu, Y., & Wei, D. (2004). Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L., *in vitro*. *Journal of Agricultural and Food Chemistry*, *52*, 5032-5039. https://doi.org/10.1021/jf049571r