PHYTOCHEMICAL AND ANTIOXIDANT ANALYSIS OF METHANOLIC EXTRACTS OF FOUR AFRICAN INDIGENOUS LEAFY VEGETABLES

Elias K. Mibeï¹*, Nelson K. O. Ojijo², Simon M. Karanja¹, Johnson K. Kinyua¹.
¹Biochemistry Department, Faculty of Science, Jomo Kenyatta University of Agriculture and Technology, Kenya
P.O. Box 62000-00200, Nairobi.
² Department of Food Science, Faculty of Agriculture, JKUAT, Kenya.
* E-mail: elimibeï@yahoo.com

Abstract
The present study investigated the phytochemical composition and relative antioxidant activity of selected African indigenous leafy vegetables (ILVs): Corchorus olitorius (Jute mallow), Crotalaria ochroleuca (Slender leaf), Solanum scabrum (Black nightshade) and Cleome gynandra (Spider plant). The crude extracts were prepared by methanol extraction. The study revealed the presence of a wide array of phytochemicals including alkaloids, flavonoids, tannins, saponins, steroids and phenols whereas terpenoids, steroids and anthraquinones were absent in most ILVs. The antioxidant activity was achieved by screening the leaf extracts for their free radical scavenging properties using diphenyl picryl hydrazyl (DPPH) and ascorbic acid as standard antioxidant. The ability of the extracts to scavenge DPPH radicals was determined spectrophotometrically at 517nm. The four ILV extracts had significant radical scavenging effects and almost all reported a significantly higher percentage of DPPH inhibition than ascorbic acid (P < 0.05). The extracts of C. olitorius and C. gynandra were most effective since they had higher percentages of radical scavenging activity and lower IC₅₀ values (concentration which scavenged 50% of the DPPH radicals). The results therefore indicated that these vegetables are phytochemical rich and natural antioxidants with potent antioxidative activities. This is of health or nutraceutical significance and thus authenticates their usefulness for medicinal purposes.

Key words: African leafy vegetables, 1, 1 diphenyl-2-picrylhydrazyl, radical scavenging activity, antioxidants.

Submitted: 25.11.2011 Reviewed: 16.01.2012 Accepted: 12.03.2012

1. INTRODUCTION

Increasing evidence suggests that majority of the bioactive phytochemical components in plants impart physiological activities and may offer a variety of health benefits such as antioxidant, antibacterial, anti-inflammatory or anticancer activity (Anupam et al., 2008). African Indigenous Leafy Vegetables (ILVs) are indigenous or traditional vegetables whose leaves, young shoots and flowers are edible (Orech et al., 2005). They have been used by communities in African countries for a long time as indispensable constituents of human diets. The species used and the wealth of indigenous knowledge vary with the culture, economic pursuits, species availability and level of influence by modernization. However, many vegetable crops are mainly consumed for their nutritional values without much consideration for their medicinal importance and very few species have been explored for chemical and biological studies (Abukutsa-Onyango, 2007).

Studies on chemical composition of ILV have shown that, they contain appreciable amounts of micronutrient content, several agronomic advantages and economic value. They also possess compounds that are essential for their medicinal values, human well-being, productive and healthy lifestyle. Most of them are used as medicine and the list of traditional vegetables used as folk medicine is long (Kimiywe et al., 2006). Some of the vegetables are also reported to cure more than one illness. Their medicinal values are believed to be dictated by their phytochemical and other chemical constituents (Fallah et al., 2005).

Epidemiological evidence indicates that, the putative benefits of a high intake of vegetables on the risk of diseases of aging may not be exclusively due to these antioxidants but other
phytochemicals contained in vegetables (Knekt et al., 1997). Growing demand for dietary antioxidant sources has triggered the search for newer, economical, nutritional and multifunctional sources possessing free radical scavenging potential (Anand et al., 2007). This calls for innovation in selecting the best sources, therefore efforts aimed at elucidating the levels of bioactive components in many plants and vegetables have to be expended in studies (Edeoga et al., 2005). Keeping in view the importance of the valuable indigenous vegetables, the present study was undertaken with the aims of evaluating the phytochemical composition and antioxidant activity of leaves of four different species of ILVs commonly consumed in Kenya. The knowledge of the bioactive components of the selected ILVs will help identify vegetables with good potential and is paramount as it adds urgency to the search for new infection-fighting strategies.

2. MATERIALS AND METHODS

2.1 Plant material
Experiments were carried out on leaves of the following indigenous vegetables; Corchorus olitorius (Jute mallow), Crotalaria ochroleuca (Slender leaf), Solanum scabrum (Black nightshade) and Cleome gynandra (Spider plant) commonly cultivated in Kenya (Figure 1).

The seeds of these ILVs were collected from KARI gene bank and planted in JKUAT farm. Leaves were harvested and processed by shade drying. Dried leaves were powdered and stored in dry airtight containers in the dark awaiting extraction.

a) Spider Plant (Cleome gynandra)
b) Jute mallow (Corchorus olitorius)
c) Slender leaf (Crotalaria ochroleuca)
d) Nightshade (Solanum scabrum)

Fig. 1: Pictures of the four target Indigenous Leafy Vegetables
2.2 Extraction of Plant Material
Initial methanol extraction was applied for the solar and shade dried samples. Fifty grams of the powdered plant material in a flask was covered with 500ml methanol and allowed to stand for 48 - 72 h. It was then filtered through Whatman filter paper No. 1 and distilled using rotary evaporator (Bibby Sterilin Ltd, RE 100B, UK) at 60°C until methanol free solid powder was obtained. The resulting extracts were then subsequently labeled as methanol extracts and preserved at 5°C in airtight bottles until further use.

2.3 Phytochemical analysis
Qualitative analysis was carried out to ascertain the presence of the different phytochemicals as described by Harborne, (1998). All chemicals used in the study were analytical grade (Sigma-Aldrich, St. Louis, MO, USA).

2.3.1 Determination of alkaloids
Two grams of the extract were extracted by warming it for 2 minutes with 20ml of 1% sulphuric acid in a 50ml conical flask on a water bath, with intermittent shaking. It was then centrifuged and the supernatant pipetted off into a small conical flask. One drop of Meyer’s reagent was added to 0.1ml supernatant in a semi-micro tube. A cream precipitate indicated the presence of alkaloids.

2.3.2 Determination of flavonoids
Five milliliters of dilute ammonia solution was added to a portion of the aqueous filtrate of the extract followed by addition of concentrated H$_2$SO$_4$. A yellow coloration observed indicated the presence of flavonoids. The yellow coloration disappeared on standing.

2.3.3 Determination of tannin
Tannin was determined by the Folin-Denis colorimetric method described by Kirk and Sawyer (1998). About 0.5 g of the dried powdered samples was boiled in 20ml of water in a test tube and then filtered through Whatman No. 42 filter paper. A few drops of 0.1% ferric chloride were added. A brownish green or a blue-black coloration indicated the presence of tannins.

2.3.4 Determination of phenolic compounds
Ferric chloride test were carried out where the extract were diluted to 5ml with distilled water. To this, a few drops of neutral 5% Ferric chloride solution were added. A dark green or a blue-black color indicated the presence of phenolic compounds.

2.3.5 Determination of steroids
Two ml of acetic anhydride were added to 0.5 g ethanolic extract of each sample with 2ml H$_2$SO$_4$. The color change from violet to blue or green in some samples indicated the presence of steroids (Harborne, 1998).

2.3.6 Determination of saponin
About 2 g of the powdered sample were boiled in 20ml of distilled water in a water bath and filtered. Ten milliliters of the filtrate was mixed with 5ml of distilled water and shaken vigorously to form a stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.3.7 Test for terpenoids
Five milliliters of each extract was mixed with 2ml of chloroform, and concentrated sulphuric acid was then carefully added to form a layer. A reddish brown coloration that formed at the interface indicated presence of terpenoids (Harborne, 1998).

2.3.8 Test for anthraquinones
Powdered plant material was boiled with 10% HCl for a few minutes, then filtered and allowed to cool. This was then partitioned against equal volume of chloroform. Formation of rose-pink color upon addition of 10% aqueous ammonium solution indicated the presence of anthraquinones (Harborne, 1998).

2.4 Determination of antioxidant activity
The radical-scavenging activity was determined using diphenyl picryl hydrazyl (DPPH) radical according to Ayoola et al.
2008. This provides information on the reactivity of the test compounds with a stable free radical and gives a strong absorption band at 517 nm in the visible region. The following concentrations of the extracts were prepared, 0.05, 0.1, 0.5, 1.0, 2.0 and 5 mg/ml in methanol in cuvette placed in the spectrophotometer (Analar grade). Vitamin C was used as the antioxidant standard at the same concentrations as the extract. One ml of the extract was placed in a test tube, and 3ml of methanol added followed by 0.5ml of 1 mM DPPH in methanol. The mixture was shaken vigorously and left to stand for 5 min. A blank solution was prepared containing the same amount of methanol and DPPH. The absorbance of the resulting solution was measured at 517 nm with a UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan). All tests were run in triplicate and the radical scavenging activity was then calculated using the following formula:

\[
\% \text{ inhibition} = \left( \frac{Ab - Aa}{Ab} \right) \times 100
\]

Where: \( Ab = \) absorption of the blank sample  
\( Aa = \) absorption of the extract.

3. RESULTS AND DISCUSSIONS

3.1 Phytochemical analysis

The study revealed the presence of a wide array of phytochemicals including alkaloids, flavonoids, tannins, saponins, steroids and phenols (Table 1). Flavonoids, alkaloids, saponins, phenols and amino acids were most common and present in almost all the ILVs, whereas terpenoids, steroids and anthraquinones were absent in most ILVs.

3.2 Antioxidant Activity

Figure 2 illustrates the absorbance versus concentration curve of methanol extracts of ILV leaves compared with ascorbic acid which is a standard antioxidant. The decrease in absorbance as the concentration increase was taken as a measure of the extent of radical scavenging activity.

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Flav</th>
<th>Alka</th>
<th>Sap</th>
<th>Tan</th>
<th>Phe</th>
<th>Anth</th>
<th>Ster</th>
<th>Terp</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. olitorius</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>C. ochroleuca</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. gynandra</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>S. scabrum</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>


![Fig. 2: Changes in absorbance of methanolic extracts of ILVs with concentration (mg/ml).](image-url)
The scavenging effects of all extracts on DPPH radicals increased as the concentration increased in the range of 0.05 – 5.0 mg/ml and it was remarkable, especially in the case of C. 

olitorius and C. gynandra (Figure 3). However, the increase in activity was only marginal when the extract concentrations were higher than 1 mg/ml. Almost all the fractions showed significantly higher 

(P < 0.05) percentage of DPPH inhibition than ascorbic acid.

Table 2 presents the IC 

50 values (the concentration which scavenge 50% of the DPPH radicals) and the maximal extent of the radical scavenging activity. The IC 

50 values of the extracts ranged from 0.01±0.00 mg/ml to 0.19±0.07 mg/ml and the maximum percentage inhibition ranged between 64–93 % with C. 

gynandra, C. olitorius, C. ochroleuca and S. scabrum extracts reporting 92.8%, 90.4%, 86.9% and 87.8%. This was much higher than that of ascorbic acid, a standard antioxidant (64.7%). On the other hand, the IC 

50 value of ascorbic acid was low indicating strong antioxidation of the ILVs.

### Table 2: IC 

50 and maximum percentage inhibition values for the ILV extracts

| Plant species | IC 

50 (mg/ml) | Maximum inhibition(%) | Concentration (mg/ml) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C. olitorius</td>
<td>0.01±0.00</td>
<td>90.4±2.98</td>
</tr>
<tr>
<td>C. ochroleuca</td>
<td>0.18±0.04</td>
<td>86.9±1.33</td>
</tr>
<tr>
<td>C. gynandra</td>
<td>0.04±0.00</td>
<td>92.8±1.12</td>
</tr>
<tr>
<td>S. scabrum</td>
<td>0.19±0.05</td>
<td>87.8±2.62</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.01±0.00</td>
<td>64.7±0.98</td>
</tr>
</tbody>
</table>

The results are the means of IC 

50 and maximum percentage inhibition values of two replicates ± SEM and their respective concentrations. SEM= Standard error of the mean. IC 

50 value - the concentration, which scavenged 50% of the DPPH radicals.

### 4. CONCLUSION

Because prevention is a more effective strategy than treatment for chronic diseases, a constant supply of phytochemical-containing plants with desirable health benefits beyond basic nutrition is essential in reducing the risk of such diseases in humans. The study authenticated the usefulness of the identified vegetables for medicinal purposes. The importance of these phytochemicals is their presumed ability to inhibit carcinogenesis. They play a variety of roles such as antioxidants, suppressors of...
tumor growth, antimutagens, enzyme modulators, chemical inactivators, and free radical scavengers. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. This therefore, justifies the therapeutic activity against a wide array of diseases and their antioxidant, antihypertensive, anti-diabetic and anti-ulcer properties that can prevent a number of diseases. With these health benefits, there is need to emphasis a diet rich in indigenous green leafy vegetables to promote health and prevent diseases in the population.

5. ACKNOWLEDGMENT

The authors thanks Biochemistry and Food Science and Technology (FST) department, JKUAT, for providing facilities to carry out the work successfully, Prof. Abukutsa-Onyango and Kenya Agricultural Research Institute (KARI) for the provision of ILV seeds and finally to Commission for Higher Education, Kenya for the grant that enabled execution of the project.

6. REFERENCES


