Antioxidant and Lipoxygenase Inhibitory Activity of *Crassocephalum crepidioides* (Benth.) S. Moore; an Underutilized Vegetable from Nigeria

*Bello Oluwasesan M. 1,2 Abiodun B. Ogbejejana 1 and Uduma A. Uduma.1*

**Abstract**

West Africa region i.e. Nigeria is blessed with many local and leafy vegetables which are known for managing some ailments beside their rich nutrient content. Some of these vegetables are tagged exotic while many are neglected or underutilized. *Crassocephalum crepidioides* is among the latter, it is reputed for the management of many ailments traditionally. This study is to evaluate the inflammatory inhibitory and antioxidant activities of the leaves of *C. crepidioides* through 2, 2′-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging and anti-lipoxygenase activities. The positive control employed are indomethacin and ascorbic acid, the IC50 value for the anti-inflammatory activity of the extract is significant (39.59 µg/mL) when compared with that of the positive control 31.06 µg/mL (Indomethacin), the extract showed a good antioxidant effect with an IC50 of 45.78 µg/mL, ascorbic acid was used as a positive control (41.56 µg/mL). This work affords new awareness on the use of *C. crepidioides*, a medicinal plant commonly used in many countries of West Africa as wild vegetable. The phytochemistry of the vegetable is largely unknown except for few screening done by few authors. Further isolation and more pharmacological evidences should be encouraged on this plant.

**Keywords**: phytochemistry; *C. crepidioides*; ABTS; anti-lipoxygenase; neglected vegetable

**Introduction**

Many medicinal plants mainly the exotic vegetables are predominately eaten for their nutritional advantages without much concern for their medicinal significance. These vegetables have various varieties which can be wild or cultivated in rural areas by the locals. The preference of these vegetables been used as food largely depend on the current and major migration of people from rural areas to the cities which is the age of civilization. This gradual loss of genetic diversity of vegetables deprives man of the opportunity to meet the future and even present challenges of vegetable production for the enhancement of health of the individual. Herbs have usually constituted the main repository of drugs and many have been known not to pose any threat to human life. They, apart from healing, provide the necessary nutrients for health and development of the human body (Bello et al., 2017). *Crassocephalum crepidioides* is also called thickhead, fireweed, Okinawa spinach, and red flower rag-leaf in English. The Yoruba tribe in South-West of Nigeria calls it ‘Efo Ebolo or Ebire’, the Japanese call it “Benibana borogiku” (Burkill, 1995; Tomimori et al., 2012). Its use is widespread in many tropical and subtropical regions, but is especially prominent in tropical Africa and it is also wildly distributed in the Okinawa Islands in Japan (Tomimori et al., 2012).
It has also been widely cultivated in Asia due to its medicinal and nutritional properties, it is well-known among folks in Asia as remedy for acute hepatitis and fever (Yoko Aniya et al., 2005). It occurs in moist areas, natural grass land, riverbanks, wastage places, on roadside and backyard gardens that are rich in organic matters (Arawande et al., 2013). C. crepidioides has been reported as effective in the treatment of indigestion (in Southern Nigeria), treatment of stomach upset, treatment for fresh wound (in Uganda), the decoction of the leaf is used in Nigeria for the treatment of headache. In Tanzania, a mixture of the leaf sap of C. crepidioides and Cymbopogon giganteus is used orally and externally for the treatment of epilepsy (Entaz, et al., 2016). Also in Tanzania, the dried leaf powder is applied as a snuff to stop nose bleeding and smoked to treat sleeping sickness. Tannin found in the roots of the plant is used to treat swollen lips (Adams, 1963).

The purpose of this study was ascertain the anti-inflammatory and antioxidant activities of C. crepidioides, an underutilized wild vegetable in Nigeria. To the best of our knowledge this is the first assessment of anti-inflammatory activity of this wild vegetable plant.

**Material and Methods**

**Collection and Preparation of Plant Materials**

Fresh green plants of *Crassocephalum crepidioides* was obtained in December, 2016 from ‘Oja- Oba’ market in Ilorin, in Kwara State of Nigeria located in the rain forest zone on latitude 10o 00’ North of the Equator and longitude 8o 00’ East of the Greenwich Meridian. The plant was identified and authenticated at Plant Biology Department, University of Ilorin. The authenticated plant materials were air-dried at ambient temperature for two weeks to completely remove the moisture content and to effectively prepare the plants for the next stage of preparation. After drying, the dried leaves were crushed into fine powder using a ceramic pestle and mortar and the samples were kept in an air tight plastic container.

**Preparation of Extracts**

Powdered *Crassocephalum crepidioides* (215.34 g) were macerated in 3 L of n-hexane in extraction jar such that the level of the solvent was above that of the plant materials. The macerated mixtures were then left for 72 hours at ambient temperature. The extracts were filtered out from the macerated mixture using Whatman 185 µm filter paper. The n-Hexane extracts were concentrated in a vacuum Rotary Evaporator under reduced pressure and suitable temperature, transferred to appropriately labelled 250 ml beaker and allowed to stand at ambient temperature to permit evaporation of residual solvents. The procedure was repeated using methanol after the residue of the n-hexane extract has been air-dried.

**Determination of 2, 2′-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity.**

The 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonate, ABTS radical cation decolourization assay based on the scavenging of ABTS•+ radicals by antioxidants component of the extracts was used. The assay follows the procedure of Atolani et al., (2013), with slight modifications (Atolani et al., 2013). All analysis was determined in duplicate.

**Anti-inflammatory Activity of the Extracts**

This activity was studied using the anti-lipoxygenase activity of the plant extract. Lipoxigenases are members of a family of non-heme iron-containing dioxygenases that catalyse the addition of molecular oxygen to polyunsaturated fatty acids in lipids containing a cis, cis-1,4-pentadiene system to give an unsaturated fatty acid hydroperoxides. It has been found that the lipoxygenase products play a key role in many inflammatory diseases (Steinhilber, 1999). The anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme. Test samples were dissolved in 0.25 ml of 2 M borate buffer at pH 9.0 and added 0.25 ml of lipoxidase enzyme solution (20,000 U/ml) then incubated for 5 min at 25°C. After which, 1.0 ml of lenoleic acid solution (0.6 mM) was added, and thoroughly mixed.

\[
\text{% inhibition} = \frac{(A_{\text{max}} - A_{\text{sample}})}{A_{\text{max}}} \times 100
\]

Where; \( A \) = absorbance

The absorbance was measured at 234 nm. Indomethacin was used as reference standard and the percent inhibition was also calculated using equation 1 above (Shinde et al., 1999).

**Analysis of Data**

GraphPad Prism 3 software (San Diego, USA) was used to determine the IC50 on through a non-regression analysis. The IC50 was taken as the concentration of sample that scavenged 50 % of the
radicals. Results are presented as mean ± standard error
of the mean.

**Result and Discussion**

Table 1: ABTS Activity of Methanol Extract of
*Crassocephalum crepidioides*

<table>
<thead>
<tr>
<th>S/N</th>
<th>Samples</th>
<th>Concentration (µg/mL)</th>
<th>% Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol Extract of C. crepidioides</td>
<td>100</td>
<td>13.36 ± 1.45</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>24.30 ± 1.74</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>34.35 ± 1.27</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>55.12 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>58.53 ± 0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ascorbic acid</td>
<td>100</td>
<td>19.65 ± 0.61</td>
</tr>
<tr>
<td>7</td>
<td>200</td>
<td>22.61 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>300</td>
<td>27.12 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>400</td>
<td>28.34 ± 0.94</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>500</td>
<td>23.35 ± 0.13</td>
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</table>

The antioxidant activity (ABTS) and 5-Lipoxygenase
inhibitory effect of the methanol extract of the leaves
of Crassocephalum crepidioides are shown in Table 1
and 3. These were carried out with in vitro methods at
various concentrations (100, 200, 300, 500) of the
extract. The extract tends to display a significant
antioxidant activity at 100 µg/mL concentration, this
was noticed with the positive control too. The higher
the concentration the less the antioxidant effect that
was noticed though there was a climax at 400 µg/mL
concentration. The IC50 value for both antioxidant and
anti-inflammatory activities of the extract can be said to
be remarkable and favourably compared (45.78 µg/mL,
39.59 µg/mL) with the positive control with IC50
values (41.56 µg/mL, 31.06 µg/mL). The positive
control is employed to determine the extent of the
significance of the extract used. Ng et al., 2012 reported
that the methanolic extract of the leaves of C.
crepidioides exhibited significant antioxidant activity
(90.04 %) when compared with other vegetables i.e.
Etlingera elotior (89.23 %), Monochoria vaginalis
(88.85 %) and Limnophila aromatoides (76.91 %)
respectively, Amin et al. (2006) also reported the
favorably higher antioxidant activity of C. crepidioides
when compared with other four Amaranths species
(Ng et al., 2012; Amin et al., 2006). Further in vitro
methods of antioxidant activity i.e. superoxide anion,
hydroxyl radicals and stable radical of 1,1-diphenyl-2-
picrylhydrazyl, have been employed to justify the
antioxidant activity of water extracts of C. crepidioides
(Tomoyuki et al., 2005). Our result reaffirms the antioxidant potential of
C. crepidioides though from the study ABTS method
was employed. The results from this study suggest that
leaves extract of C. crepidioides is a possibly valuable as
a chemopreventive and chemotherapeutic agent, thus
validating additional study of other possible beneficial
pharmacological effects.

Table 2: IC50 Anti-inflammatory Activity of Methanol Extract of
*C. crepidioides*

<table>
<thead>
<tr>
<th>Test materials</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract of C. crepidioides</td>
<td>45.78</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>41.56</td>
</tr>
</tbody>
</table>

Conclusion

From this study, it could be concluded that methanol
extract of the leaves of C. crepidioides has significant
anti-inflammatory and antioxidant activity. These
results suggest that *C. crepidioides* is an interesting plant
for the development of novel anti-inflammatory agents
So, the traditional use of *C. crepidioides* and its
pharmacological importance beside basic nutritional values is supported by this study. However, further isolation work is recommended to know its constituents and the structural activities relationship of the isolated compounds. This vegetable could play significant protective role against diseases after thorough clinical examination of the isolates from this medicinal plant. The plant can be planted more in household because of its many advantages and can be used as a health food.

Conflicts of Interest
The authors declare no conflict of interest.

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References


