RECORDSOF CHEMICAL SCIENCES

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

Bello Oluwasesan M. [1, 2], *Abiodun B. Ogbesejana* [1] *and Uduma A. Uduma.*[1]

Accepted: 17th Sept, 2019
Published: 10th Nov, 2019

1. Dept. of Applied Chemistry, Federal University Dutsin-Ma, Katsina, Nigeria

2. Dept. of Chemistry, University of Ilorin, Ilorin, Kwara State.

**Corresponding Author*
Bello Oluwasesan M. Ph.D
obello@fudutsinma.edu.ng

FRsCS Vol.1 No. 2
Official Journal of Dept. of Applied Chemistry, Federal University of Dutsin-Ma, Katsina State.
http://journal.fudutsinma.edu.ng/index.php/rcs/index
ISSN: 2705-2354 Print
2705-2362 Electronic

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 crepidoides* 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-lypoxygenase 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-lypoxygenase; 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-lypoxygenase activity of the plant extract. Lipoxygenases 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 lypoxygenase 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 0C. After which, 1.0 ml of lenoleic acid solution (0.6 mM) was added, and thoroughly mixed.

$$\% \text{ inhibition} = \frac{(A_{control} - A_{sample}) \times 100}{A_{control}} \quad \text{..... (1)}$$

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*

S/N	Samples	Concentration (µg/mL)	% Mean ± SEM
1	Methanol Extract of *C. crepidioides*	100	13.36 ± 1.45
2		200	24.18 ± 1.74
3		300	34.25 ± 1.27
4		400	38.26 ± 0.34
5		500	38.53 ± 0.57
	Ascorbic acid		
6		100	19.65 ± 0.61
7		200	22.61 ± 0.38
8		300	27.12 ± 6.03
9		400	28.34 ± 4.96
10		500	23.55 ± 0.15

Table 2: IC_{50} Antioxidant Activity of Methanol Extract of *C. crepidioides*

Test materials	IC_{50} (µg/mL)
Methanol extract of *C. crepidioides*	45.78
Ascorbic acid	41.56

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 elotoir (89.23 %), Monochoria vaginalis (88.85 %) and Limnophila aromaticoides (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). Tomoyuki et al., 2005 reportedly isolated isochlorogenic acids, kaempferol glycoside and quercetin as the active constituents from methanolic extract of the leaves of C. crepidioides with strong free radical scavenging action (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: Lipoxygenase Activity of Methanol Extract of Crassocephalum *crepidioides*

S/N	Samples	Concentration (µg/mL)	% Mean ± SEM
1	Methanol Extract of *C. crepidioides*	100	57.53 ± 0.00
2		200	55.48 ± 0.00
3		300	49.00 ± 0.00
4		400	49.40 ± 0.00
5		500	45.91 ± 0.00
	Indomethacin		
6		100	28.02 ± 0.00
7		200	44.84 ± 0.00
8		300	74.34 ± 0.00
9		400	85.32 ± 0.00
10		500	95.30 ± 0.00

Table 2: IC_{50} Anti-inflammatory Activity of Methanol Extract of C. crepidioides

Test materials	IC_{50} (µg/mL)
Methanol extract of *C. crepidioides*	39.59
Ascorbic acid	31.06

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.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Adams, C. D. (1963); Flora of West Tropical Africa. Crown Agents for Oversea Governments and Administrations, London, United Kingdom, pp. 225-297.

Amin, I., Zamaliah, M. M., Chin, W. F. (2004) Total Antioxidant activity and phenolic content of selected vegetables. Food Chemistry 87: 581-586.

Arawande, J. O.; Komolafe, E. A.; Imokhuede, B. (2013); Nutritional and phytochemical compositions of fireweed (Crassocephalum crepidioides). Journal of Agricultural Technology, 9 (2): 439-449.

Atolani, O., Olatunji, G. A., Fabiyi, O. A., Adeniji, Adekunle J., and Ogbole, O. O. (2013); Phytochemicals from Kigellia pinnata leaves shows antioxidant and anti-cancer potentials on human cancer cell line. Journal of Medicinal Food, 16, pp 878–885. http://dx.doi.org/10.1089/jmf.2012.0249.

Bello O. M., A. A. Zaki, I.S. Khan, P.S. Fasinu, Z. Ali, I.A. Khan, L.A. Usman, O.S. Oguntoye (2017)

Assessment of selected medicinal plants indigenous to West Africa for antiprotozoal activity. South

African Journal of Botany, 113;12 doi.org/10.1016/j.sajb.2017.08.002

Burkill, H.M. (1995). The useful plants of west tropical Africa 2ed. Royal Botanical Garden, Kew, London, pp. 160-164.

Entaz, B.; Maknoon, S.S.; Bashutosh, N.; Hyonok, Y. (2016); Evaluation of In vitro Antioxidant and In vivo Antihyperlipidemic Activities of Methanol Extract of Aerial Part of Crassocephalum crepidioides (Asteraceae) Benth S Moore. Trop J Pharm Res.,15 (3): 481.

Koh Tomimori, Shinji Nakama, Ryuichiro Kimura, Kazumi Tamaki, Chie Ishikawa and Naoki Mori (2012). Antitumor activity and macrophage nitric oxide producing action of medicinal herb Crassocephalum crepidioides. BMC Complementary and Alternative Medicine 12:78.

Ng, X. N., Chye, F.Y., Mohd I, A. (2012). Nutritional profile and antioxidative properties of selected tropical wild vegetables. International Food Research Journal. 19(4), 1487-1496.

Robert, O.B. (1955); The Typification of Crassocephalum moench and Gynura cass. Kew Bulletin, London, 10 (3): 455-465.

Shinde, U.A.; Kulkarni K.R.; Phadke A.S.; Nair A.M.; Dikshit V.J.; Saraf M.N. (1999); Mastcell stabilizing and lipoxygenase inhibitory activity of Cedrus deodara (Roxb.) Loud wood Oil. Indian J. Exp. Biol, 37(3): 258-261.

Steinhilber, D. (1999). 5-Lipoxygenase: a target for anti-inflammatory drugs revisited. Curr. Med. Chem., 6: 69-83.

Yoko Aniya, Tomoyuki Koyama, Chika Miyagi, Maanami Miyahira, Chiho Inomata, Shizuka Kinoshita, Toshiol Chiba (2005). Free Radical Scavenging and Hepatoprotective Actions of the Medicinal herb. Crassocephalum crepidioides from the Okinawa Islands. Biology and Pharmaceutical Bulletin 28(1), 19-23.