TOTAL PHENOLICS, FLAVONOIDS AND ANTIOXIDANT ACTIVITY OF *Icacina trichantha* Tuber Extracts

ISSN: 2141 – 3290
www.wojast.com

BASIL N ITA AND UDOH, I. E.
Department of Chemistry, University of Uyo, Nigeria.
+234 80 2334 8014; basil_ita@yahoo.com

ABSTRACT

This study investigated the phenolic and antioxidant activities of ethylacetate, ethanol and aqueous extracts of *Icacina trichantha* tubers which is used traditionally to treat diseases. The antioxidant activity was investigated by determining its 2, 2 diphenyl, 1- picryl hydrazyl (DPPH) radical scavenging activity, metal chelating potential and reducing power. The ethanol extract contained higher levels of total phenolics and flavonoids (81.41 ± 3.51 mgGAE/g and 47.44 ± 2.45mgQE/g respectively) than the other extracts. The ethanol extract also showed the highest DPPH radical scavenging ability (IC_{50} = 43.73± 1.99µg/mL) and reducing power (IC_{50} = 52.61 ± 2.01µg/mL), while the aqueous extract had the strongest ability to chelate transition metal ions (IC_{50} = 39.6 ± 1.17µg/mL).These results suggest that *I. trichantha* tubers are rich sources of natural antioxidants that can be exploited to remedy oxidative stress associated diseases and support its use in traditional medicine.

INTRODUCTION

The family Icacinaceae comprises t 23 genera and 160 species, of which *Icacinatrichantha* is a member. This plant grows abundantly in the wild in Nigeria, particularly in the rainforest zone and is used as food, medicine and fuel. Traditionally, its tuber (though reported to contain alkaloids and benzophenone) is used to treat constipation, poisoning, malaria and induce emesis (Monday and Uzoma, 2013; Shagal and Kubmarawa 2013), while the leaves are used to wrap processed oil bean seeds. Numerous biological activities have been reported for organs of this plant. Uterine contractile activity, antimicrobial, antioxidant, toxicity, purgative, anti inflammatory, hepatoprotective, antinephrotoxic activities as well as its effect on the nervous system have been reported.(Akinwumi *et al*., 2011; Okieimen *et al*., 2018; Udeh and Nwaehujor, 2011; Otun *et al*., 2015; Asuzu and Ugwueze, 1990; Asuzu *et al*., 1999; Shagal and Kubmarawa, 2013; Timothy and Idu, 2011; Asuzu and Abubakar, 1995a, b; Oke and Hamburger, 2002). Also, phytochemical pmpounds and the antinutritional factors of its tubers have been reported (Umoh, 2013; Mohammed and Dimas, 2013; Shagal *et al*., 2014). Furthermore, cytotoxic primaranes, 17- nor primaranes and their derivatives as well as stearolic acid, oleic acid and erucic acid have been reported(Zhao *et al*., 2015a, b; Otun *et al*., 2015; Monday *et al*., 2014; Guo *et al*., 2016).

Oxidative stress arising from an imbalance between endogenous/exogenous antioxidants and free radical production in the body can damage vital biomolecules such as cell lipids, proteins and DNA, and have been implicated in the etiology of diseases such as cancer, diabetes, ulcer, gastrointestinal disorders and neurodegenerative diseases (Kumar *et al*., 2013; De-Silva *et al*., 2013). In addition, synthetic antioxidants that can effectively scavenge this free radical are reported to be toxic and mutagenic, hence the need for natural antioxidants from phyto sources becomes imperative. Despite the medicinal potentials of *I.trichantha* tubers, studies on its antioxidant potentials is limited. Onakpa *et al* (2016) reported the antioxidant effects of *I. trichantha* methanolic tuber extract. In this study, we evaluate the total phenolics, flavonoids and antioxidant activities of various organic and aqueous extracts of *I. trichantha* tubers.

MATERIALS AND METHODS

Icacina trichantha tubers were collected from the wild within Akwa Ibom State, Nigeria, identified and authenticated by a taxonomist in the Department of Botany and Ecological

Studies, University of Uyo, Uyo. The plant material was air dried and powdered. The powdered material (100g) was successively macerated with ethyl acetate, ethanol and water for 48h. The filtrates were evaporated to dryness *in vacuo* and the water extract freeze dried to obtain the ethanol extract (EE), ethylacaetate extract (EaE) and the aqueous extract (AqE) respectively. The extracts were stored in air tight container at 4^{o}C until further use. The extraction yield was noted.

Evaluation of Antioxidant potential

The antioxidant potential of the extracts was determined by evaluating its DPPH radical scavenging activity, reducing power and metal chelating potentials.

(i) Evaluation of DPPH Activity:

Precisely 1ml of each extract at varying concentrations was mixed with1ml of 0.004% methanol solution of DPPH. The mixture was shaken vigorously and allowed to stand for 30 minutes at room temperature in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 517nm. The procedure was repeated for the blank and control. The radical scavenging activity was calculated using the equation:

DPPH scavenging effect (%) = $[(A_{blank} - A_{sample})/A_{blank}] \times 100$.

Sample concentration providing fifty percent inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. BHA and Vitamin E were used as positive controls (Subhasree *et al*., 2009).

(ii) Evaluation of Reducing Power:

The reducing power of the extracts was determined according to the method of Oyiazu (1986). Each sample (0.1 -20mg/ml) in ethanol (2.5ml) was mixed with 2.5ml of 200mM sodium phosphate buffer (pH 6.60 and 2.5ml of 1% potassium ferricyanide) and the mixture was incubated at 50^{o}C for 20 minutes. Thereafter, 2.5 ml of 10% trichloroacetic acid (w/v) was added and the mixture centrifuged at 200g for 19 minutes. The upper layer (5ml) was mixed with 5ml of deionised water and 1ml of 0.1% ferric chloride and the absorbance measured at 700nm against a blank. A higher absorbance indicated a higher reducing power. IC_{50} value (mg/ml) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation. Ascorbic acid and BHA were used as positive controls.

(iii) Evaluation of Metal Chelating Activity:

Metal chelating activity was determined according to the method of Decker and Welch (1990) with some modifications. Briefly, 0.5ml of each extract was mixed with 0.05ml of 2m M$FeCl_2$ and 0.1ml of 5mM ferrozine. The total volume was diluted with 2ml methanol. Then, the mixture was shaken vigorously and left standing at room temperature for 10 minutes. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562nm. The percentage inhibition rate of ferrozine – Fe^{2+} complex formation was calculated using the formula:

Scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

where $A_{control}$ = absorbance of ferrozine – Fe^{2+} complex, and A_{sample} = absorbance of sample. EDTA was used as a positive control.

Determination of Total Phenolics

Concentration of total phenolics in the extracts was determined using the Folin- Ciocalteu reagent (Lister and Wilson, 2001). 0.1ml of each extract was dissolved in 0.5 ml (1/10 dilution) of the Folin-Ciocalteu reagent and 1ml of water/methanol (1:2) was added. The solution was mixed and incubated at room temperature for 1 minute. After 1 minute, 1.5ml of 20% Na_2CO_3solution was added. The final mixture was shaken and incubated for 2 h in the dark at room temperature. The absorbance of the fraction was measured at 760nm using a UV-Vis

spectrophotometer.Gallic acid was employed as the standard and the results expressed in mg gallic acid per gram (mgGAE/g).

Determination of Flavonoids

A slightly modified version of the spectrophotometric method was used to determine the flavonoid contents of the fractions. Each extract (0.1g) was mixed with 20ml of 80% aqueous methanol, then filtered with Whatman filter paper No. 42 to obtain a clear filtrate. A 0.5ml aliquot of this filtrate was taken in a test tube and 3ml of distilled water and 0.3ml of 0.5% sodium nitrite were added. The solution was mixed and allowed to stand at room temperature for 5 minute. To this solution, 0.6ml of 10% aluminium chloride was added. After 6 min, 2ml of 1M sodium hydroxide was also added. The solution was then diluted with distilled water to make the final volume up to 10ml. The absorbance was read at 510nm. Flavonoid content was calculated using a standard calibration curve prepared from quercetin. (Subhasree *et al*., 2009).

RESULTS AND DISCUSSION

Plants are good sources of bioactive compounds, including antioxidants, which act as free radical scavengers and metal chelators. These compounds can prevent the deleterious effects associated with oxidative stress (Pereira *et al*., 2016). The yield percent, total phenolics, flavonoids and antioxidant potential of *I. trichantha* tuber extracts is given in Table 1. Total phenolic content in the tuber extracts ranged from 46.55 to 81.41mgGAE/g. Flavonoid contents were lower (13.69 - 47.44mgQE/g), and was of the order: ethanol extract > water extract > ethylacetate extract. Phenolics are powerful antioxidants capable of scavenging reactive oxygen species and chelate transition metals that can initiate deleterious free radical reactions leading to oxidative stress. Generally, the content of phenolics and flavonoids in our extracts were higher that reports for *D. alata*, tuber, yacon tuber and Kohlrabi tubers (Sakthidevi and Mohan, 2013; Pereira *et al*., 2016; Yagar *et al*., 2016).

The DPPH assay is widely used to test the ability of compounds or extracts to scavenge free radicals or donate hydrogen and to evaluate the antioxidant activity of extracts. As shown in Figure1, extracts of *I. trichanta* tubers were potent free radical scavengers in a dose dependent manner. The ethanolic extract exhibited the best DPPH scavenging activity (82.61%) at 200µg.mL. Onakpa *et al* (2016) reported a 74.3% inhibition at 400µg/mL for the methanolic extract of the same plant. This difference could depend on geography, photoperiod, temperature, processing technique and extracting solvent which might affect their secondary metabolites content. IC_{50} values (Table 1) were in the order EE >AqE > EaE; however these were inferior to BHA used as the positive control. In addition, there was a positive correlation between antioxidant activity (IC_{50}) and total phenolics (R^2 = 0.9899) and flavonoids (R^2 = 0.9759). In comparison with other tubers, our values were higher than reports for *Dioscoreaalata* tubers and Yacon tubers (Sakthidevi and Mohan, 2013; Pereira *et al*, 2016). The strong DPPH radical scavenging activity of the extracts indicate their ability to donate hydrogen to free radicals, particularly to the lipid peroxide or hydroperoxide radicals, resulting in the inhibition of the propagating phase of lipid peroxidation (Sowndharajan and Kang, 2013).

In addition, the metal chelating potentials of the extracts was evaluated. Iron may form reactive hydroxyl radicals during the Fenton reaction, and thereby contribute to oxidative stress. Thus, an important mechanism of antioxidant activity is the ability to chelate transition metal ions such as Fe^{2+}. All the extracts demonstrated potent metal chelating abilities; however, this increased with extract concentration (Fig. 2). Amongst the extracts, the aqueous extract exhibited the highest metal chelating ability (IC_{50}< 40 µg/mL). Our results indicate that the extracts are capable of preventing oxidative damage by chelating Fe^{2+} ions that may catalyse Fenton type reactions or promote the decomposition of hydroperoxide (Sowndharajan and Kang, 2013).

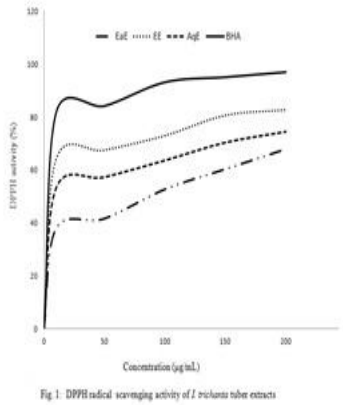

Fig 1: DPPH radical scavenging activity of *I. trichantha* tuber extracts

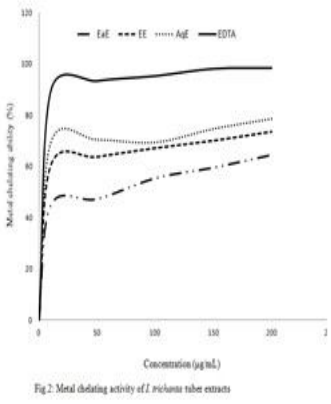

Fig 2: Metal chelating activity of *I. trichantha* tuber extracts

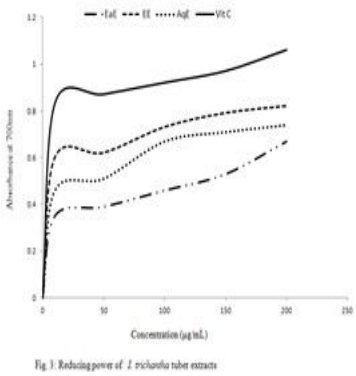

Fig. 3: Reducing power of *I. trichantha* tuber extracts

Table 1: Table 1: Total phenolics, flavonoid, DPPH scavenging, reducing power and metal chelating activity of *I.trichantha* tuber extracts.

Variables	Total phenolics (mgGAE/g)	Flavonoids (mgQE/g)	% yield	Reducing power*	DPPH activity*	Metal chelating activity*
Water extract	63.26 ± 2.12	28.11± 1.22	7.21	79.67 ± 2.42	72.72 ± 2.44	39.65 ± 1.17
Ethanol extract	81.41 ± 3.51	47.44 ± 2.45	4.29	52.61 ± 2.01	43.73 ± 1.99	59.55 ± 3.06
Ethylacetate extract	46.55 ± 1.92	13.69 ± 0.81	3.17	124.08 ± 4.55	110.42 ± 3.22	106.05 ± 2.77
Vitamin C	-	-	-	2.69 ± 0.01	-	-
BHA	-	-	-	-	2.18 ± 0.01	-
EDTA	-	-	-	-	-	< 0.1

* IC_{50} value in µg/mL is the effective concentration where DPPH radical is scavenged by 50%, ferrous ion is chelated by 50% and the absorbance is 0.5 for reducing power. IC_{50} was obtained using the regression equation.

Furthermore, the reducing power of the extracts was evaluated. Studies have shown that reducing power may serve as an indicator of potential antioxidant activity as antioxidants are also regarded as reductants and inactivator of oxidants (Sowndharajan and Kang, 2013). Like the DPPH assay, the extracts exhibited promising reducing power in a dose dependent manner (Fig. 2). The ethanolic tuber extract exhibited the strongest reducing potential (< 53 µg/mL). bserved order was: EE > AqE > EaE. Onakpa *et al* (2016) reported a ferric reducing antioxidant power of 6.7µM at 800µg/mL for the methanolic tuber extract of *I. trichantha*. The strong reducing power of the ethanolic extracts reveals that the plant is rich in phenolic compounds capable of donating electrons.

CONCLUSION AND RECOMMENDATION

Icacina trichantha tubers is rich in total phenols and flavonoids and their concentration varied with the extracting solvent. Antioxidant assay revealed that the extracts could reduce oxidative stress through their ability to scavenge DPPH radical, chelate transition metals and act as reductants. Overall, the ethanolic extract showed great promise and therefore be used for fractionation and isolation of the active principles responsible for the observed activity.

REFERENCES

Akinwumi, K. A., Odunola, O. A and Olashore, H. O (2011). The toxicity of methanolic extract of *Icacina trichantha* leaves on Wister rats. *Journal of Food Agriculture and Environment*, 9(2): 145-147.

Asuzu, I. U and Abubakar, I. I (1995a). The antihepatotoxic and antinephrotoxic activities of *lcacina trichantha* tuber extract. *Journal of Herbs, Spices and Medicinal Plants*, 3:9-20.

Asuzu, I. U and Abubakar, I. I (1995b). The effects of *lcacina trichantha* tuber extract on the nervous system. *Phytotherapy Research*, 9:21-5.

Asuzu I. U, Sosa, S and Loggia, R. D (1999). The anti inflammatory activity of *Icacinatrichantha* tuber. *Phytomedicine* 6(4): 267-272.

Asuzu, I. U and Ugwueze, E. E (1990). Screening of *Icacina trichantha* extracts for pharmacological activity. *Journal of Ethnopharmacology*, 28:151-156.

De - Silva, J.K., Cazarin, C.B.B., Colomeu, T.C., Batista, A.G., Meletti, L. M. M., Paschoal, J. A.R., Junior, S. B., Furlan, M.F., Reyes, F. G. R., Augusto, F., Junior, M. R. M and Zollner, R (2013). Antioxidant activity of aqueous extract of passion fruit (*Passifloraedulis*) leaves: *In vitro* and *in vivo* study. *Food Research International*, 53:882-890.

Decker, E. A. and Welch, B. (1990). Role of ferritin as lipid oxidation catalyst in muscle food. *Journal of Agricultural and Food Chemistry.* 38: 674-678.

Guo, B., Onakpa, M. M., Huang, X. J., Santarsiero, B. D., Chen, W.L., Zhao, M., Zhang, X.Q., Swanson, S.M., Burdette, J.E and Che, C. T (2016). Di-nor- and 17-nor-pimaranes from *Icacina trichantha. Journal of Natural Products*, 79(7):1815-1821.

Kumar, V., Lemos, M., Sharma m and Shriram, V (2013). Antioxidant and DNA damage protecting activities of Eulophia nuda Lindl. *Free Radicals and Antioxidants* 3:55-60.

Lister, E., and Wilson, P. (2001). *Measurement of Total Phenolics and ABTS Assay for Antioxidant activity* (Personal Communication). Lincoln, New Zealand: Crop Research Institute, pp 67-69.

Mohammed, H. S and Dimas, K (2013). Antimicrobial and Phytochemical Screening of *Icacina trichantha. American Journal of Biomedicine and Life Sciences*, 1(2):37-40.

Monday, O.M and Uzoma, A.T (2013). Histological changes and antidiabetic activities of *Icacina trichantha* tuber extract in beta cells of alloxan induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine*, 3(8): 628-633.

Monday, O. M., Zhao, M., Gödecke, T., Chen, W. L., Che, C.T., Santarsiero, B. D., Swanson, S. M and Uzoma, A. I (2014). Cytotoxic (9βH)-pimarane and (9βH)-17-norpimarane diterpenes from the tuber of *Icacina trichantha. Chemistry and Biodiversity*, 12:1914-1922.

Oke, J. M and Hamburger, M.O (2002). Screening of some Nigerian medicinal plants for antioxidant activity using 2,2 diphenyl picryl hydrazyl radical. *African Journal of Biomedical Research*, 5:77-79.

Okieimen, E. E., Owolabi, O.J., Ijekowa, O and Jonathan, E. M (2018). Uterine contractile activity of extracts of *Icacinatrichantha* on albino non –pregnant rat uterus. *Tropical Journal of Natural Products Research* 2(2):118-121.

Onakpa, M.M., Ode, J. O., Ajabgonna, O. P and Asuzu, I.U (2006). *In vivo* and *in vitro* antioxidant effects of *Icacinatrichantha* tuber extract. *NigerianVeterinaryJournal*, 37(3): 148-154.

Otun, K,O., Onikosi D, B., Ajiboye, A. A and Jimoh, A. A (2015). Chemical composition, antioxidant and antimicrobial potentials of *Icacina trichantha* Oliv. leaf extracts. *International Journal of Tropical Medicine,* 10 (4-6): 21-29.

Oyiazu, M. (1986). Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*.44: 307-315.

Pereira, J. A. R., Teixeira, M. C., Saczk, A. A., Barcelos, M. P., Firmino de Oliveira, M and César de Abreu, W (2016). Total antioxidant activity of yacon tubers cultivated in Brazil *Ciência e Agrotecnologia*, 40(5):596-605.

Sakthidevi, G and Mohan, V. R (2013). Total phenolic, flavonoid content and *in vitro* antioxidant activity of *Discorea alata* I tuber. *Journal of Pharmaceutical Sciences and Research*, 5(8):115-119.

Shagal, M. H and Kubmarawa, D (2013). Antimicrobial and phytochemical screeenng of *Icacina trichantha. American Journal of Biomedical and Life Sciences*, 1(2): 37-40.

Shagal, M. H.,Makhtar, H., Akinterinwa, A and Ndahi, J. A (2014). Phytochemical investigation of methanolic extract of *Icacinatrichantha* tuber. *International Journal of Current Microbiology and Applied Sciences*, 3:907-911.

Sowndhararajan, K and Kang, S.C (2013). Free radical scavenging activity from different extracts of leaves of *Bauhiniavahlii* Wright and Arn. *Saudi Journal of Biological Sciences*, 20:319-325.

Subhasree, B., Baskar, R., Keerthana, R. L., Susan, R. L., and Rajasekran, P. (2009). Evaluation of antioxidant potential in selected green leafy vegetables. *Food Chemistry* 115: 1213 -1220.

Timothy, O and Idu, M (2011). Preliminary phytochemical and in vitro antimicrobial properties of aqueous and methanol extracts of *Icacina trichantha* Oliv. Leaf. *International Journal of Medicinal and Aromatic Plants*, 1:184-188.

Udeh, N. E and Nwaehujor, C.O (2011). Antioxidant and hepatoprotective activities of ethylacetate leaf exract of *Icacina trichantha* on paracetamol induced liver damage in rats. *Continental Journal of Animal and Veternary Research*, 3: 11-15.

Umoh, E. O (2013). Anti-nutritional factors of false yam (*Icacina tricachantha*) flour. *International Journal of Food Safety*, 15:78-82.

Yagar, H., Islilir, S. S and Akagun, G (2016). Antioxidant activity of Kohlrabi leaf and tuber. *Journal of Agriculture and Life Sciences*, 3(2): 55-65.

Zhao, M., Onakpa, M.M., Bernard, D., Santarsiero, W.L., Chen, K. M., Ramamurthy, S., Swanson, S. M., Burdette, J.E and Che, C. T (2015b). (9βH)-Pimaranes and Derivatives from the Tuber of *Icacina trichantha*. *Journal of Natural Products*, 78(11): 2731-2737.

Zhao, M., Onakpa, M. M., Santarsiero, B.D., Huang, X.J., Zhang, X. Q., Chen, J., Cheng, J. J and Longnecker, R (2015a). Icacinlactone H and Icacintrichantholide from the Tuber of *Icacina trichantha. Organic Letters*, 17:3834–3837.