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Evaluating Consumption Risk And Toxicity Index: A Case Study of *tridax procumbens.*

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Abstract

This study was carried out to determine the level of some heavy metals (minerals), and selected vitamins present in *Tridax procumbens* leaf extracts and its nutritional compositions as it is recently used in phyto-medicine for the treatment of ailments and also used as source of vegetables for human consumption. All compositions were evaluated using modified standard method of AOAC, 2006. The result revealed myriad amounts (mg/100g) of heavy metals in the order of zinc (8.21 ± 0.01) > manganese (7.02 ± 0.01) > Iron (4.02 ± 0.01) > Nickel (0.62 ± 0.01) > lead (0.43 ± 0.01) > magnesium (0.42 ± 0.01) > cobalt (0.21 ± 0.01). The result for vitamins contents were expressed in mg/100g in the order of vitamin A (15.00 ± 1.41) > vitamin B_9 (8.65 ± 0.03) > vitamin C (6.33 ± 0.02). It could be deduced that the nutritional composition of this plant must have led to its therapeutic and conventional use as a vegetable but the presence of some heavy metals could be a major health concern as bioaccumulation might lead to impairment of some vital organs in the body.

Introduction

Plants used as vegetables have been employed in development of nutraceuticals, and their effects have been commendable due to the presence of some phytochemicals, vitamins and minerals. One of such plants includes *Tridax procumbens. Tridax procumbens* is a species of flowering plant in the Aster family (Compositae), a common weed in West Africa, subregion and other tropical zones of world (Funk *et al.*, 2009). The plant is called with different names; In Igbo it is called Mbuli, in Hausa it is called Harantama, in Yoruba it is called Igbalode, in English it is called coat button. It is a semi prostate, annual, creeper herb. Stem is ascending 30-50cm height, branched, sparsely hairy, rooting at nodes. Leaves are simple, opposite, mainly 3-7cm long, 1 to 4cm wide, irregularly toothed margin, base wedge shaped, shortly petioled, hairy on both surfaces. Flowers are tubular, yellow with hairs, inflorescence capitulum. *Tridax* has two types of flower: ray florets and disc florets with basal palcentation (Bhalerao and Kelkar, 2012).

Figure 1: *Tridax procumbens*

Ikewuchi *et al.*, (2009a and b) researched on the nutritional properties of the leaves of *Tridax procumbens*, also commonly used as vegetables and for medicinal purposes, because of their

myriad of pharmacological properties. These includes but not limited to use as analgesic (Prabhu *et al.*, 2011), anti-anemic (Ikewuchi and Ikewuchi, 2013), anti-arthritic (Petchi *et al.*, 2013), anti-diabetic (Bhagwa *et al.*, 2008; Ikewuchi, 2011; Pareek *et al.*, 2008), antihypertensive (Ikewuchi *et al.*, 2011; Salahdeen *et al.*, 2004), anti-inflammatory, antioxidant (Ravikumar, 2005), antimicrobial (Yoga *et al.*, 2009), hepatoprotective (Ikewuchi, 2012). The present study reports the heavy metal and selected vitamins composition of the leaves of Tridax procumbens, and in addition discusses the bioaccumulation effect of the detected compounds.

Tridax procumbens has been adduced to have many phytochemical, mineral, and vitamins constituents of, which has resulted it its use in phyto-medicine and consumption as vegetables in villages like Abakaliki, Ebonyi State, Nigeria, likewise other African countries. However, limited concern has been placed on its potential to bio-accumulate some heavy metals such lead, nickel, cobalt which might be present in an insignificant quantity.

Specific Objectives:

1. To determine the level of minerals present in *Tridax procumbens.*
2. To determine level of some specific vitamins (A, B_9 and C) in *Tridax procumbens.*

Materials and Methods

All materials, chemical and reagent used are of analytical standard.

Collection of plant material

Fresh leaf of *Tridax procumbens* was collected at the back of Biochemistry Laboratory, Ebonyi State University, Abakaliki and was authenticated by Professor S.S.C Onyekwu of Taxonomy unit of the Department of Applied Biology, Ebonyi State University.

Preparation of Plant Material

The bulk sample was sorted to remove dried and infected plants, the rest were washed in running tap water to eliminate dirt. It was dehulled manually, spread on a Laboratory tray and shade dried for 2 weeks at room temperature. The dried sample was homogenized into powdered form using manual grinder. The ground sample was sieved through 1mm test sieve to obtain a powdered processed sample used for analysis.

Method of Mineral Determination

Minerals were estimated by the use of an Atomic Absorption Spectrophotometer using a modified standard method of AOAC (AOAC, 2006). The sample solutions in the sample bottles were analyzed for the concentration of the individual elements. Each element has specific cathode discharge lamp and this lamp was used to determine a particular element. Discharge lamp emits radiation at a wavelength specific for each element being assayed. This specificity can be obtained only from a pure sample of the element that is excited electrically to produce an arc spectrum on that element.

Determination of content Retinol (Vitamin A)

This was done using spectrophotometric using a modified standard method of AOAC (AOAC, 2006). 5g of sample was dissolved into a 30mls of absolute alcohol (ethanol), and 3mls of 5% of potassium hydroxide was dissolved into it. The mixture was boiled under reflux for 30mins and was cooled rapidly with running water and filtered. 30mls of distilled water was cooled rapidly with running water and filtered. 30mls of distilled water was added and also the mixture was transferred into a separating funnel. Three portions of 50mls of ether were used to wash the mixture, the layer as discarded and the upper layer was washed with 50mls of distilled water. The extract was evaporated to dryness and dissolved in 10mls of isoprophyl alcohol and its absorbance was measured at 320nm.

$$\text{Vitamin A (mg/100g)} = \frac{200}{w} \times \frac{au}{as} \times C$$

Where,

au	=	absorbance of test sample
as	=	absorbance of standard solution
C	=	concentration of test sample
W	=	weight of sample

Determination of Folic Acid (vitamin B_9)

This was determined spectrophotometrically using a modified standard method of AOAC (AOAC, 2006). Homogenized leaves powder (500 mg) was weighed into 100 ml volumetric flask. It was dissolved with 50 ml, 3% of disodium hydrogen orthor-phosphate and shaked for 20 minutes, and make up to mark with the same solution, and then filter.

The standard folic acid powder (28 mg) was weighed and treated as above. The filtrate (40 ml) was taken from the test and 3ml of the filtrate was taken from the standard and each into separate 100 ml volumetric flask and was made up to mark with 3% disodium hydrogen orthorphosphate. Five milliliters of both standard and test each was added into 50 ml volumetric flask and was treated for colour development as follows;

Two millilitres of KMnO4 (0.4%) was added and allow to stand for one minute, two milliliters of sodium nitrate (2%) was added. Two millilitres of 5M HCl was also added and both standard tests were shaken. Two millilitres of sulphuric acid (5%), 2 ml of sodium edadate/EDTA (5%) was added, shake and allow standing for 10 minutes. Two millilitres of Azodye (0.1%w/v) was also added and allowed to stand for 10 minutes and absorbance read at 550 nm.

Potency = AT/AS × WS/WT × 3/100 ×100/40 × Average fill weight.

AT = Absorbance of test.
AS = Absorbance of standard.
WS = Weight of standard.
WT = Weight of test.
Average fill weight = 480 mg.

Determination of Vitamin C (Ascorbic Acid)

The method employed by Hussian et al., (2006) was used. 1g of every ground sample was weighed in a very 25 ml conical flask. Then 10ml of the ethanedioic acid (0.05 M)-EDTA (0.02 M) solution was added and also the mixture allowed standing for twenty-four h, to supply the desired time interval. After 24 h, the samples were filtered through 0.45 µm Whatman paper No.1. Then 2.5 ml of every sample was transferred to a separate 25 ml volumetric brown flask, after which 2.5 ml of the acid (0.05 M)-EDTA (0.02 M) solution was added. Subsequently, meta-phosphoric acid was added separately with ethanoic acid (0.5 ml), acid (5% v/v) solution (1 ml) and ammonium molybdate solution (2 ml) in each volumetric brown flask and therefore the volume was made up to 25 ml with water. The absorbance was measured at 760 nm in a very visible Spectrophotometer.

Statistical Analysis

Statistical analysis was done using statistical program for social science (SPSS) 22.0 (SPSS, Inc. Chicago, Illinois, USA). The results of replicate measurements were presented as means ± standard deviation.

Results

Results of Mineral content of *Tridax procumbens* leaf extract.

The result of mineral content of *Tridax procumbens* showed significant high levels of minerals such as, zinc, manganese, iron and moderate levels of cobalt, nickel, lead, magnesium lower than one (1) mg/100g,

Minerals	Values(mg/100g)
Magnesium	0.42 ± 0.01
Iron	4.02 ± 0.01
Zinc	8.21 ± 0.01
Manganese	7.02 ± 0.01
Cobalt	0.21 ± 0.01
Nickel	0.62 ± 0.01
Lead	0.43 ± 0.01
Values are expressed in mean ± standard deviation of triplet determination	

Table 1: Mineral composition of *Tridax procumbens* leaf extract.

Results of Vitamin content of *Tridax procumbens* leaf extract.

The result of vitamin content of *Tridax procumbens* leaf extract indicated high levels of Vitamins A, B_9 and C as shown in table 2 below.

Vitamins	Values (mg/100g)
A	15.00 ± 1.41
B_9	8.65 ± 0.01
C	6.33 ± 0.02
Values are expressed in mean ± standard deviation of triplet determinate	

Table 2: Vitamin compositions of *Tridax procumbens* leaf extract.

Discussion

Tridax procumbens leaf extract showed high level of vitamin A as shown in table 1.This high level of vitamin A might be responsible for the use of the leaf as vegetables and neutraceticals as they as pose antioxidant property, and maintenance of good vision property. The level of vitamin C showed (6.33 ±0.02mg/100g) found to be present in *tridax procumbens* leaf extract is lower than (10.63 ± 0.00mg/100g) found in the same leaf (Catherine and Jude in 2009). It also contains high level of vitamin B_9 (8.65 ± 0.03mg/100g) which is higher than (0.60 ± 0.06), found in *Seige* leaf extract, (Odunfa, 2007), which aid in body metabolism and wound healing.

The levels of Zinc, Manganese, Iron were not significantly different from the result reported by Ikewuchi and Ikewuchi (2009) on same plant. Nevertheless, Punsiri in (2015), reported a lesser concentration of Zinc (2.7 ± 3.3) in same plant in a work conducted in Thailand. The differences in concentration could be as a result of changes in geographical locations and plant growth conditions. Zinc and Iron helps in boosting the Immune system boosting, bone formation and other properties. The concentration of magnesium is much lesser compared to the report of Ikewuchi and Ikewuchi (2009) whose magnesium concentration was (8.86 ± 2.22) on same plant. Punsiri in (2015) reported concentrations of lead (0.23 ± 0.22 %) and Nickel (0.26 ± 0.25%) which was higher than the result of this present research. It has been reported that metals such as Iron, Lead, Mercury, and Nickel have the ability to produce reactive oxygen species; thus, results in lipid peroxidation, DNA alteration, and hampered calcium homeostasis (Stohs and Bagchi, 1994; Otitoju and Onwurah, 2005). Therefore, consumption of *Tridax procumbens* leaf extract as medicine or vegetable because of its pharmacological properties might require some level of assessment in order to save the exposed or vulnerable population seeking for treatment to their ailments.

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