International Journal of Applied
And Natural Sciences (IJANS)
ISSN(P): 2319-4014; ISSN(E): 2319-4022
Vol. 5, Issue 2, Feb Mar 2016; 57-68

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

EFFECT OF DEBITTERING ON PHYTOCHEMICALS AND ANTIOXIDANT COMPOSITION OF VERNONIA AMYGDALINA AND GONGRONEMA LATIFOLIUM LEAVES

OBETA N. A. & ONWELUZO J. C.

Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

ABSTRACT

The astringent taste of *Vernonia amygdalina* (VA) and *Gongronema latifolium* (GL) leaves were de-bittered through boiling and squeeze-washing . The effect of boiling and squeeze-washing on the proximate, phytochemicals, vitamin (A,C,E) composition and *in-vitro* antioxidant activities of VA and GL leaves were evaluated. The *in-vitro* antioxidant activities of the vegetable extracts were estimated using reducing power, total phenol content and 1,1-Diphenyl-2-picryl hydrazyl radical (DPPH) inhibition. Boiling and squeeze washing significantly ($p<0.05$) reduced all the nutrients in VA and GL in a similar trend. Squeeze washing had lower values ($p<0.05$) in fibre (8.83 % and 10.80 % for VA and GL respectively) and carbohydrate (67.92 % and 64.58 % for VA and GL respectively). Both boiling and squeeze-washing drastically reduced vitamin C content (from 13.32 g/100g and 10.18 g/100g to 7.37 g/100g and 3.25 g/100g in VA and GL respectively). Phytochemicals content of both leaves showed that unprocessed VA had higher alkaloids (1.73 g/100g), tannin (0.2 3g/100g) saponin (8.02 g/100g) and phytate (0.246 g/100g) than GL. The antioxidant activity of the leaves reduced with squeezing as observed in the total phenol content, reducing power and DPPH inhibition activities. From the result it is seen that de-bittering these vegetable leaves for food preparation reduced both nutrients, phytochemicals and in vitro antioxidant activities of the leaves. the indication is that de-bittered leaves of VA and GL will not provide health benefits for which these vegetables are known for. The extract from squeezed-washing contain the necessary ingredients for health improvement**.**

KEYWORDS: Squeeze-Washing, Boiling, Astringent, nutraceutical and *in-vitro* Antioxidant

INTRODUCTION

Emphasis on the consumption of leafy vegetables is gaining greater ground now than before due to the recent knowledge of nutraceutical significance of leafy vegetables. Leafy vegetables contribute substantially to food nutrients such as vitamins, minerals, fibre and even protein especially among rural dwellers where these nutrients are not adequately supplied in normal diet (Nwanekezi & Okorie 2013). According to Enemor *et al.,* (2014). The natural vegetation/ environment around us is so richly endowed with solution to most of human nutrition and health challenges, the extent to which we discover it will have enormous implication to resolving these challenges.

Some of these nutrients found in leafy vegetables acts as antioxidants and help in reducing oxidative stress and other disease conditions. A part from the nutritive value, leafy vegetables possess non nutritive bioactive chemicals which provide protective or disease preventive properties. Such bioactive compounds called photochemical or phytonutrients

interplay with the food nutrients and dietary fibre to protect the body against sickness. Aletor and Adeogun (1995) reported that phytochemicals serve dual purposes, of reducing the absorption of some essential nutrients and protecting the body against a number of biochemical and metabolic disorders. Phytochemicals which reduces food nutrients is regarded as anti-nutrients because they interfere with protein absorption or reduce mineral availability. Tannin complex with proteins, starches and digestive enzymes to reduce nutritional values of food (Chung, *et al* 1998)

These phytonutrients hold major promise in the creation of designer foods for the dietary prevention of chronic diseases (Farnham *et al.,* 1995) but yet they are regarded as plant based toxins. Most of such compounds are bitter, acrid or astringent and therefore aversive to the consumer (Drewnowski, 1995). The demand of good taste with good health may be difficult to find. Proteins in the oral cavity of human and animal serve specific functions in identifying food components in the diets of human and animals (Chandrashekar *et al*., 2006; Chaudhari & Roper, 2010). Recognition of bitter taste and aversion to it are thought to protect the organism against the ingestion of poisonous food compounds, which are often bitter. Interestingly, bitter taste receptors are expressed not only in the mouth but also in extra oral tissues, such as the gastrointestinal tract, indicating that they may play a role in digestive and metabolic processes (Wiener *et al.,* 2012). Engineering plant foods with enhanced concentrations of chemo-preventive phytonutrients is a promising new strategy for health promotion (Nwinyi *et al.,* 2008). However, any meaningful discussion of phytonutrients and health ought to consider the bitter taste of these substances (Chung et al 1998).

Although present in very small amounts, antioxidant phytochemicals impart a perceptible bitter taste to foods which are selectively bred out of plants and routinely removed from processed foods (Roy 1990). Many food industries normally remove these compounds during plant food preparation through varieties of debittering processes (Adam & Carmen 2000). This removal of astringent compound is considered important because it is assumed that good food preparation is one which is free from trace of bitter toxin. Based on this understanding it implies that bitter phytonutrients and consumer acceptance may be incompatible. Consumers show that taste is the key influence on food selection but studies on phytonutrients and health rarely considered the bitter taste of vegetable (Bravo, 1998; Ahmad & Muktar 1999). Indeed, the low amounts of bitter plant compounds in the current diet largely reflect the achievements of the agricultural and food industries (Roy 1990). The debittering of plant foods has long been a major sensory concern for food science. Among the vegetables with astringent taste are *Vernonia amygdalina* and *Gongronema latifolium*. They have characteristic bitter taste necessitating much squeezing, washing, several soakings in water or boiling before cooking and consumption to remove the bitterness (Asawalam and Hassanali, 2006). *Vernonia amygdalina* and *Gongronema latifolium* are highly utilized and popularly consumed in southern Nigeria for soup making, as spice and in preparation of staple foods (Iwu *et al.,* 1996).

Vernonia amygdalina (VA) is a perennial plant that belongs to the Composite family (Akpaso *et al*., 2011) and is planted in homes in villages as a fence post and pot herb. It grows in many zones in Africa and is drought tolerant (Bonsi *et al.,* 1995; Akpaso *et al.,* 2011). It is cherished in Nigeria for its distinctive flavour on the dishes in which it is a component. All parts of *Vernonia amygdalina* (VA) have been used as folk medicine for treatment of wounds, malaria and diabetes.

Gongronema latifolium (*Utazi* –Igbo *Arokeke*--Yoruba) is a tropical rain forest non-woody plant from Ascelepindaceae family which produces milky or clear latex. It is found mainly in Africa and South America, with a moderate representation in Northern and South Eastern Asia (Agbo *et al.,* 2005; Eze & Nwanguma, 2013). *Gongronema*

latifolium (GL) is also known to have antioxidant, hypoglycaemic, hypolipidemic and anti-inflammatory properties (Morebise *et al.,* 2002; Ugochukwu and Babady, 2002; Ogundipe *et al.,* 2005). It is primarily used as spice, vegetable in many food preparations and in traditional folk medicine mostly in southern part of Nigeria (Akuodor *et al*., 2010; Nwangwu *et al.,* 2011). It is also reported that it contain essential oils, saponins and steriods (pregnanes) among others (Morebise *et al*., 2002; Nwangwu *et al.,* 2011). This work is therefore aimed at evaluating the phytochemicals and antioxidant compositions of *Vernonia amygdalina* and *Gongronema latifolium* leaves and the extent debittering processing methods (boiling and squeeze-washing) affected the above parameters.

MATERIAL AND METHODS

Vernonia amygdalina (VA) and *Gongronema latifolium (GL)* fresh leaves were purchased from Ahia Ogige market in Nssuka town Enugu State, Nigeria. The fresh leaves were plunked from the stem washed to remove dirt and extraneous materials. Each of the leafy vegetable was divided into three parts:-

Squeeze-Washed Sample

A 200 g portion each of fresh leaves of VA and GL were separately squeeze-washed continuously in 200ml of de-ionized water for 10 min. Squeeze washing was repeated twice with 100 ml of de-ionized water at the same time, after pressing out the water, squeezed-washed leaves were homogenized and designated as squeeze-washed (SQWVA and SQWGL) samples.

Boiled Leaves

A 200 g weight each of fresh VA and GL leaves were boiled with 400 ml of de-ionized water at 100 °C for 5mins in a glass container. After draining out the water each sample was homogenised and designated as boiled (BODVA and BODGL) sample. Fresh unprocessed VA and GL were separately homogenized and designated (UNPVA and UNPG) which served as control.

QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

Alkaloid

Alkaline precipitation gravimetric method described by Harborne, (1973) was used. A known weight (1 mg) of the sample was dispersed in 10 ml of 10 % Acetic acid solution in ethanol to form a ratio of 1:10 (10 %). The mixture was allowed to stand for 4 hr at 28 ºC. It was later filtered with a Whatman No 42 grade filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous NH_4OH until the alkaloid was precipitated. The precipitate was filtered, received in a weighed filter paper, washed with 1 % ammonia solution dried in the oven at 80 ºC for 30 min. Alkaloid content was calculated as the weight difference between weight of the precipitate and filter paper and the weight of dried filter paper before filtration.

Flavonoid

This was determined according to the method of Harborne (1973). A 5 g weight of each sample was boiled in 50 ml of 2 M HCl solution for 30min under reflux. It was allowed to cool and then filtered using Whatman No 42 filter paper. A known volume (5 ml) of the extract was treated drop wise with equal volume of ethyl acetate. The flavonoid precipitated was recovered by filtration using weighed filter paper. The weight difference gave the weight of flavonoid in the sample.

Tannins

Tannin content was determined by the Folin-Denis colorimetric method described by Kirk and Sawyer (1991). A 5 g weight of each sample was dispersed in 50ml of distilled water, shaken and allowed to stand for 30 min at 28 ºC before it was filtered through Whatman No.42 grade filter paper. A 2 ml volume of each sample extract was dispensed into a 50 ml volumetric flask and mixed with 2ml standard reagent and 2.5 ml of saturated Na_2CO_3 solution. After mixing, the content of each flask was made up to 50 ml with distilled water and allowed to incubate at 28 ºC for 90 min. The respective absorbance was measured in a spectrophotometer at 260 nm. The reagent blank was used to calibrate the instrument while the absorbance values of the samples were plotted to determine tannin content against the weight of the sample.

Anthocyanins (glycosides)

This was done gravimetrically by the method of Harborne (1973). Five gramme of each test sample was hydrolyzed by boiling in 100 ml of 2 M HCl solution for 30min. The hydrolysate was filtered using Whatman No 42 filter paper. The filtrate was transferred into a separation funnel, mixed with equal volume of ethyl acetate and allowed to separate into two layers. The ethyl acetate layer (extract) was discarded while the aqueous layer was recovered, transferred into a crucible and evaporated to dryness on a steam bath. The dried extract was rehydrated with concentrated amyl alcohol in an evaporating dish of known weight and evaporated to dryness. It was further dried in the oven at 30 ºC for 30 min and cooled in desiccators. The weight of anthocyannin was determined against the weight of the sample.

Saponin

The emulsion formed from froth and oil in the qualitative test was centrifuged (Falcon 6/300R, England, CEK- 243-010J) at 10,000 x g for 15 min and the quantity of residue was used to estimate the saponin content. The process was repeated with standard saponin and compared with the samples.

Phytate

The method of Thompson and Erdman (1982) was used. A 2 g weight of each macerated vegetable sample in a flask was added 100 ml of 1.2 % HCl and 20 ml of 10 % Na_2SO_4. The flask was shaken for 2hours on a mechanical shaker (Gallenkamp England, BKS-350-010Q). The extract was filtered using vacuum filter through Whatman filter paper No1. The filtrate (10ml) was put into a centrifuge tube, added 10ml deionised water, 12 ml of ferric chloride ($FeCl_3$) solution (2.0 g $FeCl_3.6H_2O$ +16.3 ml Conc. HCl/L) mixed, heated for 75 min in a boiling water-bath and cooled. After cooling the tube was centrifuged at 10000 × g for 15 min. The supernatant was discarded and the pellet was thoroughly washed 3times with a solution of 0.6% HCl and 2.5 % Na_2S0_4. After each washing the contents were recovered by centrifugation at (10000 × g for 10min). The pellets were washed again with 3ml of conc. HNO_3 into a 400 ml beaker. Four drops of H_2SO_4 were added to the pellet in the beaker and heated for approximately 30 min on a hot plate. Following heating 5 ml of 30 % hydrogen peroxide (H_2O_2) was added to the mixture and heating continued until bobbling ceased. The residue was dissolved in 15 ml of 3 N HCl and heated at 80 ^oC for 15 min. The solution was made up to 100 ml volume and analysed for iron using Atomic Absorption Spectro-photometer (Buck, England Model 205).

Steroids

This was determined by the method described by Okeke and Elekwa (2003). A 2 g weight of each sample was

dispersed in 100ml of freshly prepared distilled water and homogenized with a laboratory blender (Philip England/China HR 1727). The homogenate was filtered and the filtrate was eluted with normal ammonium hydroxide solution (pH 9). The elute (2 ml) of each sample was put in a test tube, mixed with 2 ml of chloroform, 3 ml of ice-cold acetic anhydride and then 2 drops of concentrated H_2SO_4 were cautiously added to the cold mixture. Standard sterol solution was prepared and treated as described above. The absorbance of standard and samples were measured in a spectrophotometer at 420nm and the steroid content of the sample determined from the standard curve.

IN-VITRO ANTIOXIDANT ACTIVITIES

Determination of Total Phenol Content

Total phenolic compounds were determined using the Folin-ciocalteu method described by Ragazzi and Veronese, (1973). One millilitre of ethanolic extract (1 g of macerated leaves in 5 ml of 95 % ethanol) of each sample was added 10.0 ml distilled water and 2.0 ml of Folin-ciocalteu phenol reagent. The mixture was allowed to stand for 5 min at room temperature. Sodium carbonate (2.0 ml) was added to the mixture, the absorbance of the blue complex was measured at 680 nm. Standard solution was prepared with tannic acid and a standard calibration curve was prepared with tannic acid. The total phenol content of the extracts was expressed as mg tannic acid equivalent/g dry weight of sample.

Measurement of Reducing Power

The reducing power of the methanol extract was determined according to the method of Yen and Chen (1995). Five millilitres of the sample extracts (1 g homogenized sample in 100 ml of methanol) and BHT were each mixed with equal volume of 0.2 M phosphate buffer, pH 6.6 and 1 % potassium ferricyanide. The mixture was incubated at 50 ^oC for 20min after which an equal volume of 1 % trichloroacetic acid (TCA) was added to the mixture and it was then centrifuged at 5000×g for 10min. The upper layer of the solution was mixed with 2ml of distilled water and 4 ml of 0.1 % of ferric chloride ($FeCl_3$) at ratio of 1:1:2 and the absorbance read at 700nm. Increased absorbance of the reaction mixture was interpreted as increased reducing power. Total reducing power was expressed as absorbance units per total phenolics per gramme of sample.

1, 1-Diphenyl-2-picryl Hydrazyl (DPPH) Radical

The free radical scavenging activity of the methanol extracts were determined *in vitro* using DPPH radical according to the method of Yamaguch *et al*., (1998). A 2 ml each of the sample extract (1 g homogenized sample dispersed in 100ml methanol) was added to 2 ml of 4 % DPPH solution in a test tube. The absorbance of the mixture was measured at 517 nm at exactly 30 sec after adding the extract. The experiment was performed (in triplicate) and percentage (%) of scavenging activity was calculated using the expression 100—[100/blank absorbance × sample absorbance].

RESULT AND DISCUSSIONS

Effect of Debittering on the Phytochemicals Composition of VA and GL

Figure 1and 2 showed the effect of debittering on the phytochemicals content of VA and GL. Squeeze washing and boiling reduced ($P<0.05$) alkaloid content of VA and GL by 29 % and 16 % in VA and 45 % and 41 % in GL, respectively. The high level of alkaloids in both UNP and BOD of VA and GL were in agreement with the values reported in literature. Onyeka and Nwambekwe (2007) reported that boiling drastically reduced the alkaloid content of VA and GL. Alkaloids work on the nervous system and are used as analgesic because they are capable of relieving pains (Okeke and

Elekwa, 2003, Akinmoladun *et al.,* 2007).

Both boiling and squeeze washing decreased Flavonoid content of VA by 29 % and 34 % and in GL by 33 % and 46 % respectively. Flavoniods are soluble in water and so are affected greatly by boiling and squeeze washing; according to Okwu (2004) flavonoids are water-soluble and are easily destroyed by boiling. Del-Rio *et al*. (1977) noted that flavonoid represent the most common and widely distributed group of plant phenolic compounds which often occur as glycosides and glycosylation. This renders the molecule more water soluble and less reactive toward free radicals. This reduction in flavonoid content implies reduction of antioxidant activities of the leaves. It has been reported that Luteolin flavonoid found in VA is more potent antioxidant than BHT (Torel 1986; Ije and Ejike 2010). As phenolic compounds, they act as antioxidants with mechanism involving both free radical scavenging and metal chelating (Erdman *et al*., 2007). They have ideal structural chemistry for free radical scavenging activities and have been shown to be more effective antioxidants *in vitro* than Vitamins E and C on a molar basis (Rice-Evan *et al.,* 1997).

Squeeze washing of both leaves reduced ($P<0.05$) the tannin content by 30 % in VA and 24 % GL. Udensi *et al.,* (2008) noted that soaking mucuna in water for 24 hours reduced tannin by 76 % suggesting that tannin can be reduced by leaching. Squeeze washing was observed to be more effective in removing tannin than boiling this may be due to pressure exacted on the leaves during squeezing which removes much of the leaves' pigments and at the same time reduced the tannin content of the leaves. Most polyphenols are water soluble and easily leach into water during squeezing and washing of leaves, especially with increased washing time and many changes of water. The reduction in tannin content during squeeze washing and boiling implies that the leaves will no longer provide healing properties associated with tannin.

Anthocyanin was low in VA and squeeze washing reduced it further by 60 %. In GL, it was reduced by about 40% in SQW samples and 39% in BOD samples. Generally, reduction of anthocyanin content through squeeze washing was expected because squeeze washing involves an abrasive action which removes the green pigment in which the anthocyanin is complexed.

Boiling and squeeze washing reduced ($P<0.05$) saponin content in both leaves but squeeze washing had more impact with a reduction of up to 85 % in VA and 83 % in GL. With the high content of saponin in the unprocessed leaves and the hypocholesterolic effect associated with saponin, it does appear logical to advocate the use of leaves in unprocessed form in order to exploit maximally the benefits of this phytochemicals (Mans *et al*., 2010). The high saponin content of VA and GL is also in agreement with the value reported by Nwanjo *et al*, (2006).

Boiling as a debittering treatment reduced phytate content of VA and GL by 16 % and 6 % and 15 % and 24 % in squeeze washed VA and GL respectively. It is generally accepted that cooking reduces phytate content in vegetables (Cordian, 2008) but the extent of reduction will be a function of cooking period. High phytate in food reduces the absorption of vitamins and minerals and this can lead to mineral deficiencies especially among children who have low mineral intake (Cordian, 2008). But according to Tarwadi and Agte (2003) phytate can also act as antioxidant in protecting oxidative stress-related disorder, and can prevent excess iron from being absorbed. Phytic acid has also been shown to have potential to lower blood glucose, reduce cholesterol and triacylglycerols which reduce the risks of cancer and heart disease. The hypoglycemic response of phytate as proposed by Thompson (1986) work by the mechanism of inhibiting amylase activity by binding with the enzyme which is Ca dependent Phytate binds to starch by hydrogen bonding or to protein portion of a starch-protein complex. This causes low starch digestibility, which is also in agreement with Latha and Kapoor (2004). Squeeze washing reduced ($p<0.05$) the level of steroid in VA and GL by 64% and 50% respectively while boiling

reduced it by 21% and 8 % respectively in VA and GL. The reduction of steroid in VA and GL with squeeze washing and boiling agreed with the report of Agte *et al.,* (1999) on the effect of processing on phytochemicals. Steroid glucosides are suspected to be one of the components that cause bitterness in vegetables (Eseyin *et al.,* 2012).

Effect of debittering Methods on Antioxidant Activities of VA and GL

Figures 3 and 4 showed the effect of debittering methods on the antioxidant activities of VA and GL. Squeeze washing reduced ($P<0.05$) the total phenol, reducing power and DPPH activities of the leaves more than boiling treatment as can be observed in Figure 3 and 4.

Most soluble antioxidants of the vegetable samples were leached into the washing water during squeeze washing and boiling in both VA and GL. Since the total phenol content of squeeze washed VA and GL reduced by 92%, the implication is that squeeze washed VA used for soup and sauce making will contain very little total phenol which translates to very low antioxidant activity in the food prepared with it. This agrees with observation of Oboh, (2005) that decrease in total phenol could be attributed to the loss of antioxidant compounds like flavoniods and anthocyanins. Tarwadi and Agte 2003 also noted that loss of vitamin C reduces total phenol by 30-40% in green leafy vegetables. Reducing power is an indicator of potential antioxidant activity (Meir *et al.,* 1995). Unprocessed VA showed a higher ($p<0.05$) reducing power (1.21/700 nm) than unprocessed GL (0.77/700nm). In both VA and GL the reducing power decreased greatly with boiling and squeezes washing. The higher reducing activities observed in the UNP VA and GL may be attributed to the high content of tannin (Pulido *et al.,* 2007). The phytonutrients are important factor in reducing power activities of leafy vegetables. This observation is in agreement with the report of Huang *et al*., (2005) that reducing power work by different mechanisms in the body in preventing chain initiation, binding of transitional metals, decomposition of peroxides and enhancing radical scavenging activities.

1, 1-Diphenyl- 2-picryl Hydrazyl Radical (DPPH) has been widely used in the determination of antioxidant activity of plant extracts (Katalinic *et al.,* 2006). The radical is reduced by antioxidants through the donation of proton forming the reduced DPPH with colour change from purple to yellow. DPPH % inhibition was also observed to be lowest in squeeze washed samples with VA showing 14.30 % inhibition and GL 18.34 % as against the UNP (49.2 % and 53.7 % for VA and GL respectively). This shows little presence of free radical scavengers implying poor source of antioxidant activity (Anyasor & Ogunwenmo 2010).

CONCLUSIONS

Boiling for 5min and squeeze washing drastically reduced most phytonutrients (alkaloids, flavoniods, tannins, saponin and phytate) to a very low level that means low antioxidant activity. These effects on the nutrition and phytonutreints indicates that the use of these processed vegetables only will lead to malnutrition and reduction in health benefits for which these vegetables are known for.

RECOMMENDATION

A more scientific method of reducing bitterness in these vegetables without affecting their active ingredients should be investigated. Moreover these active ingredients of the leaves should be isolated and purified to get a pure compound for bioassay. The rate of absorption of vitamins and phytochemicals in the body and the effect on the body organs should be investigated in detail.

REFERENCES

1. Abiodun O. A. and Adeleke, R. O. (2010). Comparative studies on nutritional composition of four melon seeds varieties. Pakistan Journal of Nutrition, 9(9): 905–908.
2. Agbo, C. U.; Baiyeri, K. P. and Obi, I. U. (2005). Indigenous knowledge and utilization of Gongronema latifolia Benth: a case study of women in University of Nigeria, Nsukka. Bio-Research Journal, 3 (2): 66–69.
3. Akpaso, M. I. Atangwho,I. J. Akpantah, A. Fischer,V. A. Igiri, A. O. and Ebong, P. E.(2011). Effect of Combined Leaf Extracts of *Vernonia amygdalina*(Bitter Leaf) and *Gongronema latifolium* (Utazi) on the Pancreatic β-Cells of Streptozotocin-Induced Diabetic Rats.British Journal of Medicine and Medical Research 1(1): 24-34.
4. Akuodor, G.L. IdrisUsman, M.S. Mbah, C.C. Megwas, U.A. and Akpan, J.I (2010). Studies on anti-ulcer analygesic and antipyretic properties of the ethanolic leaf extract of *Gongronema latifolium* in rodents. African Journal of Biotechnology 9:2316-2321.
5. Aletor, M.V.A. and Adeogun, D.A. (1995). Nutrient and anti-nutrient components of some tropical leafy vegetables. Food Chemistry 53:375-379.
6. Asawalam, E.F and Hassanali, A. (2006).Constituents of the essential oil of *Vernonia amygdalina* as maize weavil protectants. Tropical and subtropical agro- ecosystem 6:95-102.
7. Bonsi, M.L.K., Osuji, P.O., Tuah, A.K. and Umunna, N.N. (1995). *Vernonia amygdalina* as a supplement to left straw fed to Ethiopian sheep. Agroforestry systems 31(3): 229—241.
8. Bravo, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. Nutritional Review 56:317-333.
9. Burkill, H. M. (1985). The Useful Plants of West Tropical Africa: Review. Dalziel's J.M Royal Botanic Gardens, Kew. 2nd Edition.
10. Chandrashekar J, Hoon M.A.; Ryba N.J. and Zuker C.S. The receptors and cells for mammalian taste. Nature. 2006; 444:288–294.
11. Chaudhari N, Roper S. D. (2010). The cell biology of taste. Journal Cell. Biol. 190:285–296.
12. Cordain, L. (2008). Healthy Eating Politics.http://www.healthy-eating-politics.com healthy- eating –guide. Html. Accessed 20th December 2012.
13. Del-Rio,A., Obdulio, B.G., Castillo,J., Martin R.R. and Ortuno,A. (1977). Uses and properties of Citrus flavonoids. Agriculture and Food Chemistry. 45:4505-4515.
14. Drewnowski A. and Rock C.L (1995). The influence of genetic taste markers on food acceptance. American Journal Clin Nutr; 62:506–511.
15. Drewnowski, A. and Gomez-Carnerors, C. (2000). Bitterness, phytonutrients and the consumer: A review. The American Journal of clinical Nutrition. http://ajcn.nutrition.org/. Accessed 1st July 2015.

16. Eseyin A.O., Igboasoiyi, C.A, Igbo C, Igboasoiyi, A, Ekarika J and Dooka B (2012). Effects of leaf extract of Vernonia amaygdalina on the pharmacokinetics of Dihydroartemisinin in rat. Pharmacologia 3:713-718.
17. Farnham M.W.; Simon P.W.; Stommel J.R... (1999). Improved phytonutrient content through plant genetic improvement. Nutr. Rev: 57: S19–26.
18. Harborne, J.B.(1973). Phytochemical methods: A guide to modern technique of plant analysis. Chapman and Hall Ltd. London pp 49-188.
19. Huang, D.J; Chen, H.J; Lin,C.D. and Lin Y.H. (2005). Antioxidant and antiproliferative activities of water spinach constituents. Botanical bulletin of Academia Sinica 46:99-106.
20. Iwu, M.M. Okunji, E. and Akah, P.A.(1996). The hypoglycaemic principles of *Dioscorea dumentorium*. Plant Medicine. 229(2): 163-169.
21. Katalinic, V. Milos,M.T.Kulisic, T. and Jukie, M. (2006). Screening of 70 medical plants extracts for antioxidant capacity and total phenols. Food Chemistry 94:550-557.
22. Kirk, R.S. and Sawyer, R. (1998). Pearson composition and Analysis of Foods. 9th Ed. Longman Scientific and Technical England Pp 607-617.
23. Latha, N. and Kapoor, R. (2004). Chemical composition and post prandial glycaemic effects of some plant Gum. Journal Food Science and Technology41 (1): 83-85.
24. Mans, G.W. Zhang, Y., Huang, L. and Liu, C. (2010). Chemical study and medical application of saponin as anti cancer agents. Fitoterapia 81(7): 703-714.
25. Meir, S.J., Kanner, B.A. and S. P. Hadas, (1995).Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. Journal of Agriculture Food Chemistry 43: 1813-1817.
26. Morebise, O., Fafunso, M.A., Makinde, J.M., Olajide, O.A. and Awe, E.O. (2002). Inflammatory property of the leaves of *Gongronema latifolium.* Phytother Research 16:75-77.
27. Nwanekezi, E.C and Okorie, S.U. 2013 effects of holding methods and time on the vitamin contents of five tropical leafy vegetables. Nig. Food Journal 31: 53-63.
28. Nwangwu, S., Ofusoru, D.A., Josiah, S., Amegor, O.F., Njoya, H. and Ayoka, A.O. (2011). The effect of aqueous and ethanolic leaf extracts of Vernonia amygdalina on some vital organs in adult wistar rats. Journal of Cell and Molecular, biology 9(1):75-82
29. Nwanjo, H.U, Okafor, M.C. and Oze, G.O.(2006). Antilipid peroxidative activity of *Gongronema latifolium* in streptozotocin induced diabetic rats. Nigeria Journal of physiological Science. 21(1-2): 61-65.
30. Nwinyi, O. C. Chinedu, N. S. and Ajani, O. O. (2008). Evaluation of antibacterial activity of Pisidium guajava and Gongronema Latifolium, Journal of Medicinal Plants Research 2 (8): 189–192.
31. Oboh, G. (2005). Effect of blanching on the antioxidant property of some tropical green leafy vegetable. Food Science and Technology. 38(5):513-517.
32. Ogundipe, O.O., Moody, J.O. Akinyemi, T.O and Raman, A. (2005). Hypoglycemic potentials of methanolic

extracts of selected plant foods in alloxanized mice. Plant Food for Human Nutrition58 (3): 1-7.

33. Okeke, C.U. and Elekwa, 1. (2003). Phytochemical study of the extract of *Gongronema Latifoluim Benth* . Journal of Health and visual science 5 (1): 47-55.

34. Okwu, D.E. (2004). Phytochemical and vitamin content of indigenous spices of south Eastern Nigeria. Journal Sustain Agricultural Enviroment 6:30-34.

35. Onyeka, E.U. and Nwambekwe, I.O. (2007). Phytochemical profile of some leafy vegetable in South East Nigeria. Nigeria Food Journal 25(1): 67-77

36. Osuagwu, G.C.E. (2008). The effect of rate of application of poultry manure on the phenol flavonoids and steroids potentials of the leaves of *Ocimum gratissium*. Journal of sustainable Agriculture and Environment 10 (2):106 – 111.

37. Pulido, R., Bravo, L. and Saura, C. F.(2007). Antioxidant activity of dietary polyphenols as determined by a modified Ferric reducing/antioxidant power assay. Journal of Agriculture Food Chemistry 48:3396-3402.

38. Ragazzi, E. and Veronese, G. (1973). Quantitative analysis of phenolics compounds after thin-layer chromatographic separation. Journal of Chromatography **77**: 369-375

39. Rice-Evans, C.A. Miller, N.J. and Paganga, G. (1997). Antioxidant properties of phenolic compounds. Trends in Plant Science 2:152-159.

40. Roy G. M. (1990). The applications and future implications of bitterness reduction and inhibition in food products. Food Sci Nutr; 29:59–71.

41. Sabinus O. O. Eze and Nwanguma, B. C (2013). Effects of Tannin Extract from Gongronema latifolium Leaves on Lipoxygenase Cucumeropsis manii Seeds. Journal of Chemistry http://dx.doi.org/10.1155/2013/864095. Accessed 26th Jan. 2015.

42. Tarwadi, K and Agte, V (2003). Potential of commonly consumed green Leafy vegetables for their antioxidant capacity and its linkage with the micronutrient profile. International Journal of Food Science and Nutrition 54(6):417-425.

43. Thompson, D.B. and Erdman (JR), J.W (1982). Phytic acid determination in soybeans. Journal of Food Science 47:513 -514.

44. Torel, J., Cillard, J. Cillard, P. (1986). Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochem., 25: 383-385.

45. Trease, G.E and Evans, M.C. (1983). Pharmacognosy 12th edition Bailliere Tindall London pp 343-383.

46. Udensi, E. A, Arisa, N.U and Maduka, M. (2008). Effect of processing methods on the levels of some Antinutritional factors in *Mucuna flagellipes.* Nigerian Food Journal 26(2): 53-68.

47. Ugochukwu, N.H. and Babady, N.E. (2002). Antioxidant effects of Gongronema Latifolium in hepatocytes of rat models of non insulin dependent diabetes mellitus. Fitoterapia 73:612-618.

48. Ugochukwu, N.H.; Babady, N. E. Cobourne, M.K. and Gasset, S.R. (2003). The Effect of *Gongronema*

49. Wiener, A.; _Shudler, M; L. _Levit, A. and Niv, M. (2012) BitterDB: a database of bitter compounds. Nucleic Acids Research 40: 413-419

50. Yamaguchi, T. Takamura, H. Matoba, T. and Terao, J. (1998). HPLC method for evaluation of the free radical scavenging activity of foods by using 1, 1 –diphenyl-2-picryl hydrazyl. Biological Science Biotechnology and Biochemistry 62:1201-1204.

51. Yen, G.C. and Chen, H.Y. (1995). Antioxidant activity of various tea extracts in relation to their anti-mutagenicity. Journal Agricultural Food Chem. 43 (1): 27–32.

APPENDICES

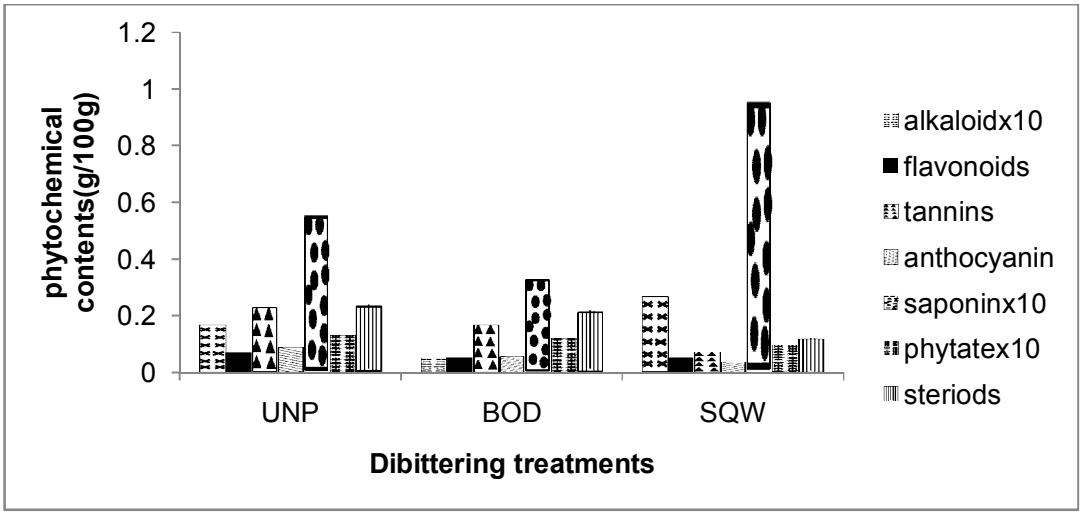

Figure 1: Effects of debittering Treatments on phytochemicals Content of *Vernonia Amygdalina*

UNP – unprocessed, BOD – boiled, SQW – squeezed washed.

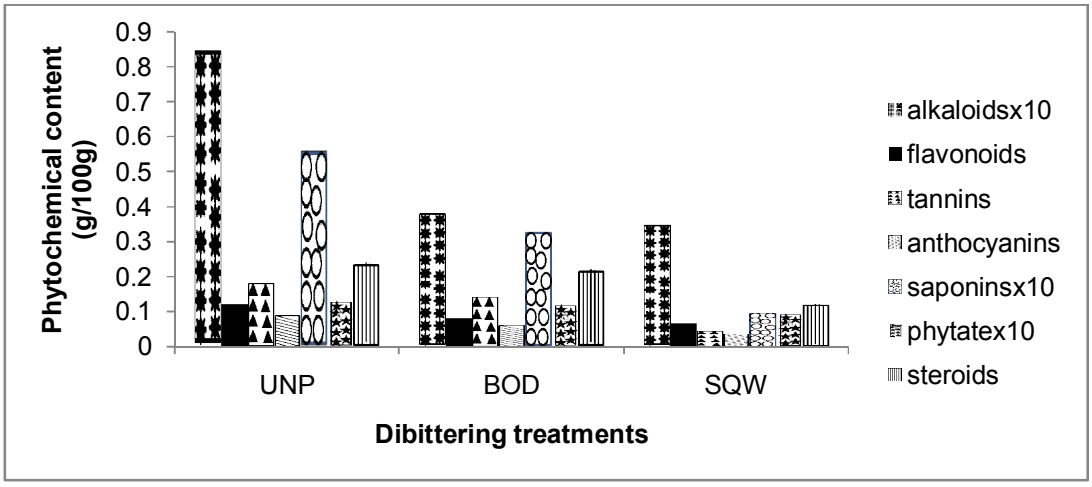

Figure 2: Effects of debittering Treatments on phytochemicals Content of *Gongronema Latifolium*

UNP – unprocessed, BOD – boiled, SQW – squeezed washed.

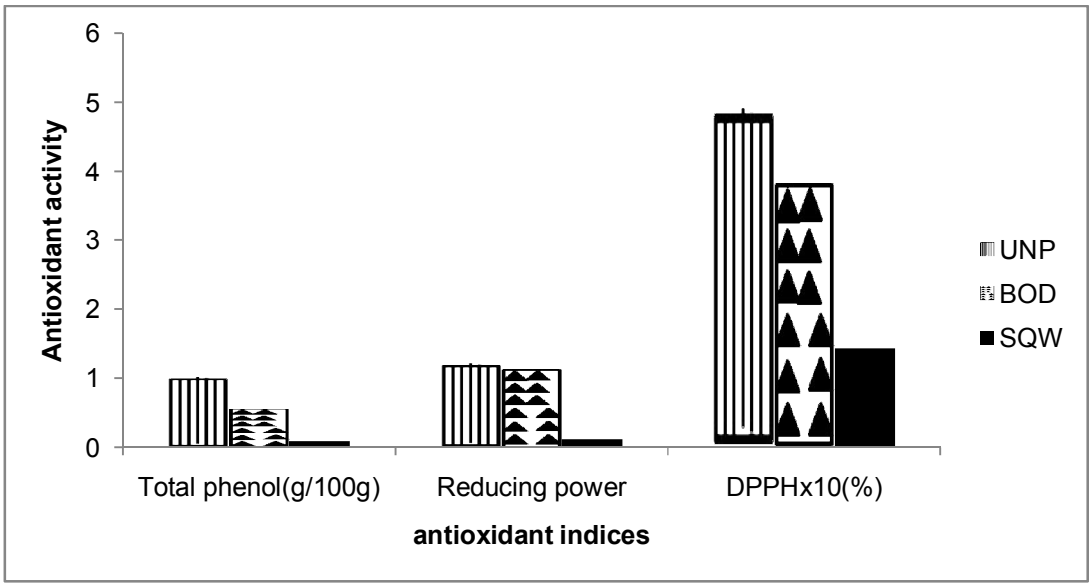

Figure 3: Effect of debittering Methods on Antioxidant Activities of *Vernonia Amygdalina*

DPPH = 1, 1-Diphenyl- 2-picryl Hydrazyl Radical, UNP – unprocessed, BOD – boiled, SQW – squeezed washed.

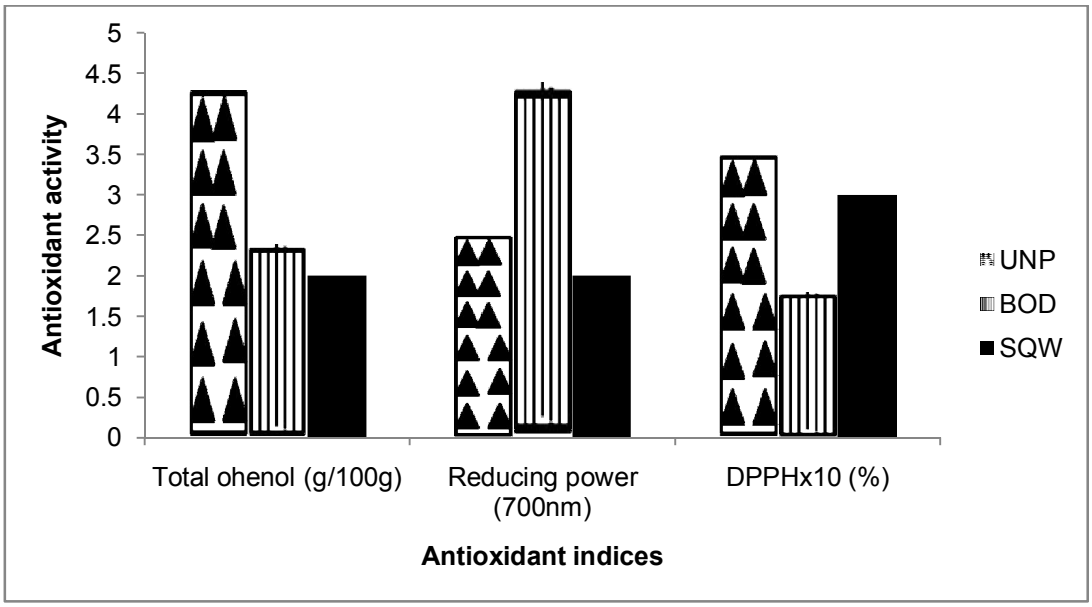

Figure 4: Effect of debittering Methods on Antioxidant Activities of *Gongronema Latifolium*

DPPH = 1, 1-Diphenyl- 2-picryl Hydrazyl Radical, UNP – unprocessed, BOD – boiled, SQW – squeezed washed.