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Comparative analysis of phytoconstituents in commonly used vegetables in gas flaring and non-gas flaring communities in southeastern Nigeria

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Abstract

A comparative study to determine the impact of gas flaring (GF) on some phytoconstituents of five commonly used green leafy vegetables was done. Two locations in south-east states in Nigeria, Ibeno in Akwa Ibom State, a gas flaring (GF) community and Nsukka in Enugu State, a non-gas flaring (NGF) community, were used. Five fresh green leafy vegetable samples were used for this study (*Amaranthus hybridus, Gnetum africanum, Talinum triangulare, Telfairia occidentalis,* and *Vernonia amygdalina*) and were obtained from community farmlands during the rainy season between August and November, 2016. After collecting and identifying the green leafy vegetables from five different farmlands in GF and NGF areas and at a distance of about 2km radius from the flare site in GF communities, detailed laboratory analysis was done for alkaloids, flavonoids, saponins and tannins. For *A. hybridus,* the flavonoid and tannin contents in NGF community were significantly ($p<0.05$) higher than in GF community; *G. africanum,* the alkaloid and tannin contents were higher in NGF community; *T. triangulare,* the alkaloid content alone was higher in NGF community; *T. occidentalis,* the alkaloid, flavonoid, saponin and tannin contents were significantly ($p<0.05$) higher in the NGF community; *V. amygdalina*, the tannin content alone was higher in NGF community compared to the GF community. Most green leafy vegetables from NGF community produced higher and better phytoconstituent concentrations than the GF community. This can be attributed to the non-pollution of the former environment.

Keywords: Analysis, Gas flaring, Green leafy vegetables, Phytoconstituents, Southeastern Nigeria

Introduction

Vegetables are herbaceous plants whose parts or whole are eaten as supporting foods or main dishes, and they may be aromatic, bitter or tasteless (Edema, 1987). Nigeria is endowed with a variety of traditional vegetables which may be in the form of leaf (pumpkin), pod (green beans), fruit (garden eggplant), seed (lentils), roots (carrots), flower (cauliflower) and bud (broccoli) and different types are consumed by the various ethnic groups for different reasons. The nutrient content of different types of vegetables varies considerably, and they are major sources of vitamins, essential amino acids, minerals and antioxidants (Fasuyi, 2006). They are included in meals for their nutritional value; however, some are reserved for the sick and convalescent because of their medicinal properties (Ogbonnia *et al*., 2008). Most vegetable leaves have great nutritional, herbal and medicinal value (Idu & Onyibe, 2007). Phytoconstituents constitute a wide range of substances that play an important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules such as carbohydrates, lipids, proteins and DNA (Atmani *et al*., 2009). It has been reported that certain phytochemicals play important roles in the antioxidant defense systems of vegetative plants (Ugochukwu and Babady, 2003). In humans and most animals, alkaloids and flavonoids have been observed to possess anti-diuretic, antispasmodic, anti-inflammatory and analgesic effects. However, they inhibit certain mammalian enzymatic activities such as those of phosphodiesterase, prolonging the action of cyclic AMP. These phytochemicals can induce actions of glucagon and thyroid-stimulating hormones even when they are not needed (Owoyele *et al*., 2002).

These vegetables contain appreciable amounts of oxalate, saponin, cyanogenic glycoside, alkaloid, tannin and phytate. These secondary metabolites are used by some plants for defense and protection (Beecher, 2003). According to the Nutrition Society, about 150 phytoconstituents have been studied in detail (Johnson, 2007), and some examples include: flavonoids, saponins, isoflavones, alkaloids, phytosterols, carotenoids, glycosides, tannins, monoterpenes, polyphenols, lignans, anthocyanins, monophenols, stylbenes, triterpenoids, xanthophylls, phenolic acids and organosulfides. They are responsible for the colour, flavor, taste and odour of plant foods (Erdman *et al*., 2007). Research has shown that phytochemicals are found in fruits, vegetables and nuts and help to slow aging process and reduce the risk of many diseases including cancer, heart diseases, stroke, high blood pressure, cataract, osteoporosis and urinary tract infection (Akah *et al*., 2002). The quantities of phytoconstituents in leafy vegetables and other plants may vary depending on their environmental conditions. Activities of oil exploration and other industries result in pollution through constant gas flare, oil spills and industrial effluents affecting aquatic and terrestrial ecosystems (Omofonmwan & Odia, 2009).

Recent studies have investigated the impact of gas flaring on micro-climate and vegetation (Efe, 2003), soil, air, and water quality (Ekanem, 2001). Other studies associated gas flaring with increasing poverty among rural women and climate change (Emerole, 2008); while flaring gas in the western countries has been minimized, in Nigeria it has grown proportionately with oil production (ERA/FOE, 2005). Flared gases constituent is mainly methane, carbon monoxide, carbon dioxide, hydrogen sulphide, sulphur dioxide, nitrogen dioxide, volatile organic compounds (VOCs) like benzene, black carbon soot, polycyclic aromatic hydrocarbons (PAHs) like methane, particulate matter (PM) and toxic heavy metals (THM) (Kostiuk *et al*., 2004). They lead to the destruction of vegetations and phytonutrients like alkaloids, glycosides, tannins or saponins, reduction in their nutritive and medicinal values, immune suppression, reduced life expectancy, varied health challenges, depletion of soil and aquatic organisms, loss of soil nutrients and inhibition of seed germination and seedling growth (WHO, 2009). Based on the uses of phytoconstituents, this study was designed to determine the impact of GF on phytoconstituents contained in fresh green vegetables when compared to the NGF area.

Materials and Methods

Plant collection and identification

Five different fresh and mature green leafy vegetable samples were collected from Nsukka Local Government Area of Enugu State, non-gas flaring (NGF) and Ibeno Local Government Area of Akwa Ibom State, gas flaring (GF), locations, both in Southern Nigeria, between August and November, 2016. About 500g of the vegetable samples were collected and they are: *Amaranthus hybridus* (african spinach)*, Gnetum africanum* (wild spinach)*, Talinum triangulare* (water leaf)*, Telfairia occidentalis* (pumpkin leaf)*, and Vernonia amygdalina* (bitter leaf). The vegetables from NGF community were collected from five different locations- Ikpa, Ogige, Beach, Onuiyi and Ibagwa farms while those from GF community were also collected from five areas-Ibeno, Ibeno/Eket boundary (IEB), Eket1, Eket2 and Eket3 farms at a distance of about 2km radius from the flare site. They were separately packaged, labeled and identified by a plant taxonomist in the Plant Science and Biotechnology Department of University of Nigeria, Nsukka. Detailed descriptions of the vegetable plants are available in literature (Akoroda, 1990; Akachukwu & Fawusi, 1995; Akachukwu, 2001; Ezekwe *et al*., 2002).

Plant processing and extraction

The fresh leafy vegetable samples were rinsed with distilled water, dried for 24 hours at room temperature on top of laboratory bench, and then pulverized into a coarse powder using pestle and mortar. The cold maceration extraction method was used for the extraction of the plant materials as described for the different phytoconstituents (Harborne, 1973; AOAC, 1990; Obadoni & Ochuko, 2001) and separated with labels for different vegetable samples from both GF and NGF communities. The yield was calculated in percentage as the weight of material obtained divided by weight of starting material multiplied by 100 over one (Harborne, 1973; AOAC, 1990; Obadoni & Ochuko, 2001) ; the crude extract was labeled according to the vegetable sample and stored in a refrigerator at a temperature of 10°C until time for use.

Determination of presence and concentration of the phytoconstituents

Total alkaloid: Three grams (3g) of each sample were weighed into 250 ml beaker and 200 ml of 20% acetic acid were added and covered with aluminum foil to stand for 4 hours. This was filtered using Whatmann No 4-filter paper and funnel, and then the extract was concentrated using a waterbath at 60°C to one quarter of the original volume. Drops of concentrated ammonium hydroxide were added to the extraction until precipitates were completely formed. The solution was allowed to settle and the precipitate was collected by filtration, dried in an oven at 100 °C to a constant weight (Harborne, 1973). The alkaloid content was calculated in percentage as:

$$\%\ \text{alkaloid} = \frac{\text{weight of filter paper and alkaloid precipitate} - \text{weight of empty filter paper}}{\text{weight of sample used}} \times \frac{100}{1}$$

Total flavonoid: Two grams (2g) of each sample were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The mixture was filtered using Whatmann No 4-filter paper. The filtrate was later transferred into a crucible and evaporated to dryness using hot air oven at 80°C and weighed (Harborne, 1973). The flavonoid content was calculated in percentage as:

$$\%\ \text{Flavonoid} = \frac{\text{weight of evaporating dish and flavonoid extract} - \text{weight of empty dish}}{\text{weight of sample used}} \times \frac{100}{1}$$

Total saponin: Ten grams (10g) of each sample were dissolved in 200 ml of 20% ethanol. The suspension was heated over a hot waterbath for 4 hours with continuous stirring at about 55°C. The mixture was filtered using Whatmann No 4-filter paper and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over waterbath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel, and 20 ml of diethyl ether were added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded and the purification process was repeated. Thereafter, 60 ml of n-butanol was added and the combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath at 60°C. After evaporation, the sample was dried in an oven at 80°C to a constant weight (Obadoni & Ochuko, 2001). The saponin content was calculated in percentage as:

$$\%\ \text{Saponin} = \frac{\text{weight of beaker and saponin extract} - \text{weight of empty beaker}}{\text{weight of sample used}} \times \frac{100}{1}$$

Total tannin: Two grams (2g) of each sample were dissolved in 50 ml of distilled water and shaken. The mixture was allowed to stand for 30 minutes at 37°C before it was filtered through Whatmann No 4-filter paper and funnel. Thereafter, 2 ml of the extract was dispersed into a 50 ml volumetric flask. Similarly, 2 ml of standard tannin solution and 2 ml of distilled water were put in separate volumetric flasks to serve as standard and 0.1 ml of Folin-Denis's reagent was added to each of the flasks, and then 2.5 ml of saturated sodium carbonate solution added. The content of each flask was made up to 50 ml with distilled water and allowed to incubate at 37°C for 90 minutes. Their respective absorbance was measured in a spectrophotometer at 760 nm using the reagent blank to calibrate the instrument to zero (AOAC, 1990). The percentage tannin was determined by the relationship:

$$\%\ \text{Tannin} = \frac{\text{absorbance of sample} \times \text{concentration of standard}}{\text{absorbance of standard} \times \text{weight of sample used}}$$

Data analysis

The results were presented in Tables as mean ± SEM and were analyzed using an independent Student's "t" test to compare the means of the two sample groups from both communities. Significance was accepted at the level of $P \le 0.05$.

Results

Table 1 shows the results of the colour, aroma, consistency and yield of the different vegetable samples from both NGF and GF locations. The total yield (22.87%) from NGF location is higher than the total yield (22.34%) from GF location. Table 2 shows the result of mean concentration of phytoconstituents of *A. hybridus* (green leaf). There is no significant (p>0.05) difference between the mean concentrations of alkaloid, flavonoid and tannin from both communities. However, saponin content in *A. hybridus* from GF community is significantly (p<0.05) higher compared to NGF community. Table 3 shows the result of mean concentration of phytoconstituents of *G. africanum* (wild spinach). The mean concentration of alkaloid from NGF community is significantly (p<0.05) higher than GF community; for flavonoid and saponin, the mean concentrations from GF area is significantly higher than NGF area. There is no significant difference (p>0.05) between the mean concentration of tannin from both communities. Table 4 shows the result of mean concentration of phytoconstituents of *T. triangulare* (water leaf). Alkaloid content from GF community is significantly (p<0.05) lower than that of NGF community, while flavonoid, saponin and tannin contents from GF community are significantly (p<0.05) higher than NGF community.

Table 5 shows the result of mean concentration of phytoconstituents of *T. occidentalis* (pumpkin leaf). The mean phytoconstituent concentration from NGF community are significantly (p<0.05) higher than GF community. Table 6 shows the result of mean concentration of phytoconstituents of *V. amygdalina* (bitter leaf). The mean concentration of alkaloid, flavonoid, and saponin from GF community is significantly (p<0.05) higher than NGF community, whereas the tannin content from both communities is not significantly (p>0.05).

Table 1: Physical characteristics of vegetables obtained from non-gas and gas flaring (NGF) community

S/N	Name of vegetable	Colour	Aroma	Consistency	Yield (%)
		Non-gas flaring community			
1	*A. hybridus*	greenish black	odourless	syrupy	3.37
2	*G. africanum*	greenish black	odourless	syrupy	6.47
3	*T. triangulare*	greenish black	odourless	syrupy	2.54
4	*T. occidentalis*	greenish black	odourless	syrupy	7.39
5	*V. amygdalina*	greenish black	odourless	syrupy	3.10
		Gas flaring community			
1	*A. hybridus*	greenish black	odourless	syrupy	4.00
2	*G. africanum*	greenish black	odourless	syrupy	5.60
3	*T. triangulare*	greenish black	odourless	syrupy	2.81
4	*T. occidentalis*	greenish black	odourless	syrupy	2.37
5	*V. amygdalina*	greenish black	odourless	syrupy	7.56

Table 2: Quantitative phytoconstituents (%) of *A. hybridus* (green leaf) from non-gas flaring and gas flaring communities

Phytoconstituent (%)	NGF (Nsukka)	GF (Ibeno)
Alkaloid	1.38 ± 0.05	1.43 ± 0.06
Flavonoid	1.73 ± 0.12	1.45 ± 0.16
Saponin	0.30 ± 0.03	0.79 ± 0.03*
Tannin	0.06 ± 0.00	0.05 ± 0.00

*Indicates a significant difference between the two communities (p<0.05). For respective phytoconstituent: Values are represented as mean ± SEM of the five vegetable plant materials

Table 3: Quantitative phytoconstituents (%) of *G. africanum* (wild spinach) from non-gas flaring and gas flaring communities

Phytoconstituent (%)	NGF (Nsukka)	GF (Ibeno)
Alkaloid	3.72 ± 0.15	1.93 ± 0.08*
Flavonoid	2.73 ± 0.07	3.26 ± 0.11*
Saponin	0.29 ± 0.04	0.58 ± 0.04*
Tannin	0.08 ± 0.00	0.08 ± 0.00

*Indicates significant difference between the two communities (p<0.05). For respective phytoconstituent: Values are represented as mean ± SEM of the five vegetable plant materials

Table 4: Quantitative phytoconstituents (%) of *T. triangulare* (waterleaf) from non-gas flaring and gas flaring communities

Phytoconstituent (%)	NGF (Nsukka)	GF (Ibeno)
Alkaloid	0.77 ± 0.10	0.01 ± 0.00*
Flavonoid	1.28 ± 0.09	2.02 ± 0.19*
Saponin	0.18 ± 0.03	0.33 ± 0.03*
Tannin	0.01 ± 0.00	0.06 ± 0.00*

*Indicates significant difference between the two communities (p<0.05). For respective phytoconstituent: Values are represented as mean ± SEM of the five vegetable plant materials

Table 5: Quantitative phytoconstituents (%) of *T. occidentalis* (pumpkin leaf) from non-gas flaring and gas flaring communities

Phytoconstituent (%)	NGF (Nsukka)	GF (Ibeno)
Alkaloid	3.84 ± 0.18	1.83 ± 0.13*
Flavonoid	3.59 ± 0.09	1.33 ± 0.14*
Saponin	0.39 ± 0.02	0.31 ± 0.02*
Tannin	0.11 ± 0.01	0.07 ± 0.01*

*Indicates a significant difference between the two communities (p<0.05). For respective phytoconstituent: Values are represented as mean ± SEM of the five vegetable plant materials

Table 6: Quantitative Phytoconstituents (%) of *V. amygdalina* (bitter leaf) from non-gas flaring and gas flaring Communities

Phytoconstituent (%)	NGF (Nsukka)	GF (Ibeno)
Alkaloid	1.30 ± 0.09	2.49 ± 0.14*
Flavonoid	1.71 ± 0.09	3.01 ± 0.38*
Saponin	0.33 ± 0.01	0.47 ± 0.02*
Tannin	0.07 ± 0.00	0.06 ± 0.00

*Indicates significant difference between the two communities (p<0.05). For respective phytoconstituent: Values are represented as mean ± SEM of the five vegetable plant materials

Discussion

The comparative analysis of the presence and concentration of phytoconstituents of common vegetables in GF and NGF areas of Akwa Ibom and Enugu States of Nigeria were conducted using standard laboratory methods. The extractive values of the vegetables showed the presence of the yields 3.37%, 6.47%, 2.54%, 7.39%, 3.10% for NGF area and 4.00%, 5.60%, 2.81%, 2.37%, 7.56% for GF area respectively of the starting materials. In the NGF area, *T. occidentalis* and *G. africanum* had the highest yield while *A. hybridus* and *V. amygdalina* gave the intermediate yield as *T. triangulare* gave the lowest yield. Conversely, in GF location, *V. amygdalina* gave the highest yield while *A. hybridus* and *G. africanum* had intermediate yield as *T. triangulare* and *T. occidentalis* maintained the lowest yield. The different yields of the plants from different locations are possibly due to pollution, which can reduce yield, varied concentrations of phytonutrients and soil compositions in the areas and also changes in the pharmacological, bioactive and anti-nutritive composition of the vegetables (Ifemeje, 2015). The colour, aroma, taste and consistency were noted to be greenish-black, odourless, tasteless and syrupy, respectively and were known to be consistent with vegetables in Nigeria (Akachukwu, 2001). The yields and concentrations of phyto compositions sampled varied from plant to plant, seasons, habitat, or geographical location (Akachukwu & Fawusi, 1995). The result of this study demonstrated that in both communities (NGF/GF), higher concentrations of the phytoconstituents alkaloids and flavonoids, were recorded than saponins and tannins. This shows the predominance of these phytoconstituents (alkaloids/flavonoids) in the five leafy green vegetables sampled for this research work. The result of this study shows that the concentrations of some phytoconstituents from NGF location like alkaloids and flavonoids, especially for *T. occidentalis*, were seen to be very high when compared to those from GF area; whereas some phytoconstituents from GF location (alkaloid and saponin), (flavonoid and saponin), (flavonoid, saponin and tannin), and (alkaloid, flavonoid and saponin) are higher than those from NGF sites. This could be due to activation of the plant nutritive and soil compositions by the surrounding heat emitted from the flare. This also suggest that vegetables from NGF area are of better ethno medicinal values and protect the body against oxidative stress, diseases and cancers (FAO, 2007). Also, the concentrations of phytoconstituents in vegetables from NGF area were greater than the concentrations from GF area. This is possibly due to the flared gas which would have damaged most phytoconstituents. Damage to the phytoconstituents is equivalent to destruction of vital nutritive components and reduction in the ethno medical potency of the green vegetables from the polluted (GF) area. This supports the statement that such flaring constitutes hazard to plant well-being, as it can affect soil composition and thus pose threat to plants metabolism by altering their nutrient and medicinal composition (Ujowundu *et al*., 2011). In this study, *G. africanum* (wild spinach) and *T. occidentalis* (pumpkin) green vegetables had higher ($p < 0.05$) levels of phytoconstituents when compared to other vegetable plants; *T. triangulare* (water leaf) green vegetable had the least ($p > 0.05$) level of phytoconstituents when compared to other vegetable plants used in the NGF area. From the results in the GF area, *G. africanum* (wild spinach) and *V. amygdalina* (bitter leaf) green vegetables had higher phytoconstituents when compared to other vegetable plants, while, *T. triangulare* (water leaf) had the least of phytoconstituents when compared to other vegetable plants. The very high levels of phytoconstituents in wild spinach and pumpkin vegetable plants are in support of their high folkloric, ethnomedicinal and nutritive values [Ezekwe *et al*., 2002). In conclusion, this study has established that most green vegetable plants from NGF area produced higher and better phytoconstituents than the GF area which is largely polluted. It is recommended that further studies should be conducted in other NGF and GF locations of Nigeria.

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Conflict of interest

The authors declare that there is no conflict of interest.

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