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Qualitative and Quantitative Evaluation of Phytochemical Contents of Ogbono and Bitterleaf Soups of Enugu State, Nigeria

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Abstract

The health benefits of phytochemical consumption have been echoed over the years. Being a plant product, sourced specially from vegetables, which are abundant in Africa, there is a likelihood of phytochemicals' presence in African dishes. To affirm or disprove this, the standard protocol was used to assess the qualitative and qualitative evaluation of the phytochemical contents of ogbono and bitterleaf soups of Enugu State, Nigeria. Recipes for these soups were collected from two urban (Nsukka and Ehamufu) and two rural (Obukpa and Neke) communities in Enugu State using Focus Group Discussion (FGD). These recipes were used to prepare the dishes, which were homogenised and screened for alkaloids, tannins, saponins, steroids, terpenoids, glycosides, flavonoids, carotenoids, and phenols. The phytochemicals present were also quantified. Phytates and oxalates were directly quantified (not screened). The Statistical Product and Service Solution (IBM-SPSS, version 22) was used to analyse the data obtained. Only Alkaloid was present in appreciable amounts (+++) in Bitterleaf soup. Glycoside, Terpenoid and Saponin were not detected in both Bitterleaf or Ogbono soups. Ogbono soup had the highest Phenol values (19600mg/100g). The concentration of Phytate was found to be the lowest and of the same values (30mg/100g) in both Bitterleaf and Ogbono soups. Soups contain minimal amounts of phytochemicals which were within tolerable limits, except for Phenol. Therefore, the consumption of these soups is encouraged.

Keywords: Traditional soups, Bitterleaf soup, Ogbono soup, Phytochemicals, Nigeria

Introduction

Phytochemicals are naturally occurring chemical compounds formed during the plant's normal metabolic processes (Okigbo et al., 2009; Webb, 2013) as primary and secondary metabolite which functions as a natural defence system for plants. They are largely responsible for the colour, flavour, aroma, and odour of plant foods; blueberries' dark hue, broccoli's bitter taste, and garlic's pungent odour (Koche et al., 2016; Ugboko et al., 2020; Ugwuona & Uchenna, 2014; Webb, 2013). Phytochemicals are also found naturally in fruits, vegetables, flowers, medicinal plants, leaves, seeds, herbs, and roots. They work synergistically with some plants' nutrients and fibres to prevent the insurgencies of diseases and more accurately guide against disease outbreaks (Akinduro et al., 2017; Okigbo et al., 2009; Olaniyan, 2016). According to Ugwuona and Uchenna (2014), both epidemiological and clinical studies have proven that phytochemicals present in cereals, fruits, and vegetables are mainly responsible for reducing the incidence of chronic and degenerative diseases among populations whose diets are high in these foods. They are health-promoting and disease-preventing. These edible disease fighters (phytochemicals) play a defensive role against major chronic diseases and other health-related complications. As a result, there has been an increased search for phytochemical constituents that possess antioxidant and antimicrobial potency in recent times (Olaniyan, 2016).

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga et al., 2005). Although mainly found in Africa, which is rich in vegetation, plants, and vegetables, it affirms the study conducted by Beaudry (2013) on the environmental treatment of the impacts of exogenous glucocorticoids on hyperinsulinemia, where he stated that the cure to almost all diseases in the world can be found in the forests of Africa. Slik et al. (2015) also stated in their work titled 'an estimate of the number of tropical tree species" that Africa has the highest estimated number of tree species with over 15,000 species, compared to other regions such as the Neotropics (Central and South America), which had an estimated 8,000 species.

Most African foods, especially Nigerian foods/soups, are prepared from plant materials that are reported to contain phytochemicals useful to the body (Otuaga et al., 2020). These phytochemicals are isolated from plants and are useful and effective for us in this era (Siddiqui & Moid, 2022). The medicinal properties associated with these plants depend largely on the bioactive phytochemical components of the plants, which have hitherto had several physiological actions on the human body (Akinduro et al., 2017). Phytochemicals, either alone or in combination, have tremendous therapeutic potential in curing various ailments. Epidemiological and animal studies suggest that the regular consumption of fruits, vegetables, and whole grains reduces the risk of chronic diseases associated with oxidative damage (Prakash et al., 2012). Without specific knowledge of their cellular actions or mechanisms, phytochemicals have been considered possible drugs for millennia. For example, Hippocrates may have prescribed willow tree leaves to

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abate fever. Salicin, having anti-inflammatory and pain-relieving properties, was originally extracted from the bark of the white willow tree and later synthetically produced to become the staple over-the-counter drug aspirin. Researchers have discovered that beta-carotene is beneficial in the healing of cancer. Carrots have been offering this nutrient for years, but they are only now getting attention. (Olaniyan, 2016). Without a doubt, the cure for almost all diseases in the world can be found in the forests of Africa.

Nigeria, the giant of Africa, has been blessed with a lot of plants and vegetables, from which most mouthwatering delicacies in Nigeria are prepared. According to Edeoga et al. (2005), medicinal plants such as *Cleome Nutidosperma, Emilia Coccinea, Euphorbia Heterophylla, Physalis Angulata, Richardia Bransitensis, Scopania Dulcis, Sida Acuta, Spigelia Anthemia, Stachytarpheta Cayennensis,* and *Tridax Procumbens*, contain different phytochemicals. Studies have also found phytochemicals in dishes/soups consumed in Nigeria. Various research conducted by Edeoga et al. (2005), Igwenyi & Azoro (2014), Obi & Davidson (2022), and Otuaga et al. (2020) not only detected phytochemicals in soups and their ingredients but also affirmed a tangible amount of phytochemicals in the soups. However, only a few studies have been carried out to detect and quantify phytochemicals in these traditional African soups, mainly consumed in Nigeria. Due to the lack of studies on the phytochemicals present in traditional soups, it has been difficult to state the health benefits of consuming other soups and encourage their consumption and connection with phytochemicals. This study, therefore, is aimed at providing empirical nutrition data on Ogbono and Bitter leaf soups to add to the body of knowledge on the nutritional benefits of these soups.

Objectives of the study: The general objective of the study was the qualitative and quantitative evaluation of phytochemicals in Ogbono and Bitterleaf soup of eastern Nigeria. Specifically, the study carried out:

1. qualitative analyses of phytochemicals in Ogbono and Bitter leaf soup
2. quantitative analyses of phytochemical Ogbono and Bitter Leaf soup

Materials and methods

Study design: Quasi-experimental study design was adopted in this study.

Procurement of materials: All the ingredients used in preparing the traditional soups were sourced from local markets in both urban and rural communities of Enugu State. Specifically, the ingredients were purchased from Ogige market and Eke Ehamufu in Nsukka and Ehamufu, (urban communities), and Elu-agu market and Ore Neke in Obukpa and Neke (rural communities).

Soup sample preparation: The traditional soup recipes were obtained from a previous study carried out in the Nsukka, Ehamufu, Obukpa, and Neke communities of Enugu State. as detailed below. The soup preparation was done in the Diet Therapy Laboratory, Home Science and Management Department, University of Nigeria, Nsukka, Enugu State.

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Recipe: "Ogbono" soup

Ingredients	Quantity
"Ogbono"	1 cup
Meat	500g
Smoked fish	250g
Crayfish	150g
water	2 litres
Ugba/Okpei (locust bean)	50g
Pepper	25g
Aja-azu (dry fish particles)	300g
Palm oil	1 level cup (milk cup)
Ora/uturukpa	50g
Salt	to taste
Bouillon cubes	4 cubes

Method of Preparation

1. Grind Ogbono separate, Okpei separate, and Crayfish and pepper together.
2. Bring water to a boil, add fish, Crayfish, pepper, bouillon cubes and other ingredients and cover to boil. add vegetables and remove from heat.
3. Place a dry pot on the fire and allow it to dry add Palm oil and Okpei and allow to melt.
4. Add Ogbono and stir together (do not talk or else the Ogbono will not draw)
5. Pour the liquid and turn it till properly mixed.
6. Allow cooking, taste, and stop cooking.

Recipe: Bitter leaf soup

Ingredients	Quantity
Bitter leaves (fresh or dried)	2 wraps
Assorted meat (beef, goat eat, pomo (cow skin), shaki and ideal)	1 kg
Cocoyam ('ede')	3-4 medium size
Crayfish	150g
Water	2 litres
Smoked fish (Optional)	2 pieces
Stockfish (optional	2-3 pieces
"Ogiri" (fermented locust bean)	1 tablespoon
Palm oil	2 cooking spoons
Ground dry pepper	2 tablespoons
Salt	to taste
Bouillon cubes	4 cubes

Method of preparation:

1. Start by boiling the meat; start with tougher meats like cow leg, and 'shaki' first. When they are slightly tender, add beef or any other softer meat. When all the meats are tender, add the Stockfish, and leave to cook till soft.
2. While the meats are boiling, boil the cocoyam with the skin on. Do not add salt, boil till tender, this should take roughly 20 minutes on medium heat.
3. When they are soft, gently peel the skin off and pound till smooth in a mortar.
4. Wash the bitter leaves with water, boil with enough water for 15 minutes afterwards and then rinse with cold water to get rid of as much bitterness as possible; there should be almost no hint of bitterness left in the leaves after washing.

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5. When the meat and fish are all soft, add crayfish, and smoked fish, then add the grounded or blended pepper and stir, add palm oil and leave for 3-4 minutes.
6. Lower the heat, add the blended cocoyam to the stock, and be careful not to add too much. The consistency should be semi-fluid.
7. The cocoyam will dissolve and thicken the soup; the thickness will depend on preference.
8. Then add the 'ogiri' and stir, taste for seasoning, and adjust if necessary. Then add the washed bitter leaves and leave to cook for 2-3 minutes. Do not overcook the vegetables.
9. Serve as desired.

Preparation of samples for chemical analysis: After cooking, the wet soup samples were homogenized, properly packaged, labelled, and taken to the Food and Nutrition Laboratory, Department of Home Science and Management, University of Nigeria, Nsukka, for qualitative and quantitative evaluation of phytochemicals.

Chemical analysis: Quantitative and qualitative evaluation of phytochemicals in all samples was done in duplicate.

Phytochemical screening: Various tests were employed to determine the presence of specific phytochemicals in the samples.

Alkaloid determination: Mayer's test, as described by Ajuru et al. (2017), was conducted. Terpenoids were detected using a test recommended by Ejikeme et al. (2014). The test for saponin was carried out by shaking the test solution with water and observing the formation of copious lather to indicate the presence of saponins.

Tannin determination: The test solution was mixed with a basic lead acetate solution, and the formation of a white precipitate confirmed the presence of tannins. For glycosides, the extract was boiled with dilute sulfuric acid, followed by the addition of chloroform and shaking. The organic layer was separated, and the gradual addition of ammonia resulted in a pink-to-red colour, indicating the presence of glycosides. For flavonoids, the test solution was mixed with magnesium turning and concentrated hydrochloric acid, then boiled. The appearance of a red or orange colour indicated the presence of flavonoids.

Phenol determination: Phenols were detected by adding ferric chloride solution to the test solution, with a bluish-green colour indicating their presence. Steroids were identified by adding chloroform, acetic anhydride, and concentrated sulfuric acid to the test solution, resulting in a purple colour that changed to blue or green. In the case of carotenoids, 1g of each sample was extracted with 10 ml of chloroform through vigorous shaking. After filtration, 85% sulfuric acid was added, and the presence of carotenoids was confirmed by the appearance of blue colour at the interface.

Quantitative analysis of phytochemicals

Alkaloid determination: Alkaloids content was determined by the alkaline precipitation-gravimetric method described by Harborne (1973).

Oxalate determination: Total oxalate in the sample was assayed using the method of AOAC (199).

Pytate and Tannin determination: Phytate and tannin were determined using the method of AOAC (2010).

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Saponin determination: Saponin was determined according to Ochuko and Obadoni's (2001) method.

Flavonoid determination: The Boham and Kocipai method (1994) was used for flavonoid determination.

Phenol determination: The method of analysis of the Analytical Methods Committee of the Royal Society of Chemistry (AMCRS) was used for phenol determination.

Terpenoids determination: To determine terpenoids, about 10g of the sample was taken and soaked in alcohol for 24 hours. It was filtered, and the filtrate was extracted with petroleum ether; this ether extract was treated as total terpenoids.

Glycoside determination: Onwuka (2005) was used to determine glycosides in this study.

Steroid determination: **One millilitre (1 ml)** of methanolic extract steroid solution was transferred into a 10 ml volumetric flask. Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5% w/v, 2 ml) were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water bath maintained at 70±2°C for 30 minutes with occasional shaking and diluted to the mark with dilute water. The absorbance was measured at 780nm against the reagent blank.

Carotenoid determination: A measured weight of each sample was homogenized in methanol using a laboratory blender. A 1:10 (1%) mixture was used. The homogenate was filtrated to take up the carotenoid, mixed well, and then treated with 20 ml of distilled water in a separating funnel. The other layer was recovered and evaporated to dryness at a low temperature (3550°C) in a vacuum desiccator. The dry extract was then saponified with 20 ml of ethanoic potassium hydroxide and stored overnight in a dark cupboard. The next day, the carotenoids were taken up in 200 ml of ether and then washed with two portions of 20 ml distilled water. The carotenoid extract (ether layer) was dried in a desiccator and then treated with light petroleum (petroleum spurt) and allowed to stand overnight in a freezer (-10°C). The next day, the precipitate steroid was removed by centrifugation, and the carotenoid extract was evaporated to dryness in a weighed evaporation dish, cooled in a desiccator, and weighed. The weight of the carotenoid was determined and expressed as a percentage of the sample weight.

Statistical analysis: Data obtained was analyzed statistically using Statistical Product for Service Solution (IBM-SPSS), version 22 and presented as Mean ± Standard deviation.

Results

Qualitative analysis

Table 1 represents the result of the qualitative analysis of phytochemicals in ogbono and bitter-leaf soups. Tannins were detected in moderate amounts in both soups, while saponin, glycosides, and terpenoids were not detected in either soup. Although flavonoids and phenol were detected in 'Ogbono' soup, they were not detected in Bitter leaf soup. Alkaloid is the only phytochemical present in appreciable amounts in Bitter leaf soup.

Table 1: Qualitative analyses of phytochemicals in the traditional soups

	Soup Sample	
Phytochemicals	**"Ogbono" soup**	**Bitter leaf soup**
Tannin	++	++
Phenol	++	-
Flavonoid	++	-
Saponin	-	-
Alkaloid	++	+++
Carotenoid	-	++
Glycoside	-	-
Terpenoid	-	-
Oxalate	++	++
Steroid	-	++

Keys: +++ = present in appreciable amount; ++ = moderately present; + = minimally present; - = not detected.

Table 2 divulges the quantified phytochemicals in both soups. The highest concentration of phenol (19.6) was detected in 'Ogbono soup'. However, certain phytochemicals such as carotenoid, glycoside, terpenoid, steroid, and saponin were not detected in 'Ogbono soup' when the phytochemicals were quantified. The lowest concentration of the phytochemical—phytate (0.03), was detected in both soup samples. Phytochemicals such as glycosides, terpenoids, and saponins were still not detected in both soups.

Table 2: Phytochemical composition of traditional soups

Phytochemicals	**Soup Sample**		
	"Ogbono" soup (mg/100g)	**Bitter Leaf Soup (mg/100g)**	**The tolerable limit for human consumption** (mg/100g)
Tannin	320	240	2500
Oxalate	330	770	920
Phenol	19600	ND	0.0001
Flavonoid	2970	2770	189700
Phytate	30	30	1000
Alkaloid	3490	3980	-
Carotenoid (mg)	ND	0.29	6
Glycoside	ND	ND	4
Terpenoid	ND	ND	0.1
Steroid	ND	9520	-
Saponin	ND	ND	420

Keys: ND = not detected;

Discussion of findings

The phytochemical screening of the 'Ogbono' soup revealed the presence of tannin, phenol, alkaloid, and flavonoid in moderate amounts but did not detect carotenoids, steroids, glycosides, terpenoids, or saponin. However, there is a disparity between this result and the results of Ezekwe et al. (2021), and Ihenetu et al. (2020) in their study on the Phytochemistry and antioxidant activity of Irvingiagabonensis (Bush mango) seed sample and Phytochemical screening, gas chromatography-mass spectroscopy studies and antioxidant property of aqueous extract of Ogbono (Irvingia gabonensis) respectively which recorded that tannins, saponins, and steroids were moderately present, flavonoids and alkaloids were minimally present, and terpenoids were not detected. Although there were discrepancies in the presence of the phytochemicals in the studies conducted by Ezekwe et al. (2021) and Ihenetu et al. (2020), the tannins, terpenoids, flavonoids, and alkaloids that were detected in their study, show a degree of similarity with the results from the current study.

The findings from Don Lawson (2018) on the phytochemical screening of *Irvingia gabonesis* (Ogbono cotyledon) revealed that tannin was present in moderate amounts; alkaloids, flavonoids, glycosides, saponin, and steroids were minimally present, and oxalate and phytate were not detected. In Don Lawson's (2018) study on the phytochemical screening of Irvingia gabonesis (Ogbono cotyledon), it was observed that tannin was present in moderate quantities. However, minimal presence was noted for alkaloids, flavonoids, glycosides, saponin, and steroids, while oxalate and phytate were not found. This present study indicated moderate amounts of tannin and did not detect glycosides, saponin, and steroids which corresponds with the findings of Don Lawson (2018) but detected alkaloids and flavonoids in moderate amounts.

This is not surprising since "ogbono soup" contains other ingredients that might have boosted some phytochemicals in them. Researchers have found that cooking and other methods of food preparation reduce the number of phytochemicals in food, which could be the reason for the reduction and absence of some phytochemicals like glycoside in the soup (Fang et al., 2022; Parlermon et al., 2023; Pellegrini et al., 2010). Another reason could be the processing undergone by the ogbono during its preparation. Sharma et al. (2023) have also reported that fermentation, soaking, roasting, washing, rubbing, and other food processing methods reduce saponin levels in foods and grains.

In the study conducted by Ali et al. (2019), the phytochemical screening and antibacterial activity of the Bitter leaf (Vernonia amygdalina) showed the presence of phenol, flavonoids, and saponins. Odukoya et al. (2019) also detected flavonoids, saponins, and terpenoids in the aqueous extract of sun-dried and freeze-dried bitter leaves. However, this contradicts the findings from this study as flavonoids, terpenoids, phenol and saponin were not detected in the bitter leaf soup. The

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contradictory results may be because Ali et al. (2019) and Odukoya et al. (2019) conducted their studies on the bitter leaf, while the present study screened for phytochemicals in bitter leaf soup. The methods used in processing and cooking the soup might have led to the loss of the phytochemicals.

The present study detected the presence of tannins, steroids, and alkaloids, which shows a similarity with the study conducted by Usunobun and Okolie (2016), which reported a minimal presence of flavonoids, saponins, tannins, steroids, alkaloids, and glycosides in their study, though differing in the amount present/detected. The present study revealed that alkaloids were present in appreciable amounts, tannin and steroids were present in moderate amounts, and saponin was not detected. The differences in the results could be because Usunobun and Okolie (2016) researched washed bitter leaf, which is the major ingredient of the soup, and not the soup itself. The presence of tannin in Bitter leaf soup could also be from the cocoyam used in its preparation and the bitterness of the bitter leaf. Tannin has an astringent and bitter taste, and the level of tannin in bitter leaves is dependent on the amount of bitterness remaining in the leaves after washing (Anacletus et al., 2015; Azuzu, 2018). Ukong et al. (2014) reported the presence of tannin in cocoyam in appreciable amounts, which could have contributed to the presence of tannin in the soup. The phytochemicals not detected in the soup could be due to the processing and cooking methods. Cooking reduces the amount of glycoside and saponin in food, while preparation and processing of food decrease its flavonoid levels (Yuk et al., 2015). Overall, the present study provides valuable insights into the phytochemical composition of bitter leaf soup and the potential health benefits it may offer.

According to the study conducted by Usman et al. (2019), the aqueous seed extract of Irvingia gabonensis (Ogbono) contains flavonoids (1.05%), glycosides (1.57%), alkaloids (0.07%), and saponins (0.32%). This study reported that Ogbono soup contains flavonoids (2.97%), and alkaloids (3.49%), and did not detect glycoside and saponin. The result of the study conducted by Anaduaka et al. (2022) stated that the aqueous pulp extract of Irvinga gabonensis contains phenol (1.23mg), steroid (0.52mg), flavonoid (6.60mg), glycoside (0.40mg), terpenoids (4.68mg), alkaloids (2.26mg), and saponin (0.90 mg). The findings of this study (phenol (19600mg), flavonoid (2970mg), and alkaloid (3490mg). while steroids, glycosides, terpenoids, and saponin were not detected) still contradict the results of the study by Anaduaka et al. (2022). All the detected phytochemicals were within tolerable limits except phenol. Phenol is known to be toxic to human health when in excess, however, this toxicity occurs when it is consumed on its own, (Jewell, 2019), which is hardly the case in soups.

The number of phytochemicals found in the bitter-leaf soup was inconsistent with the results of Ali et al. (2019), who quantified phytochemicals in bitter leaves. According to the result

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of their study, the bitter leaf contained tannin (1.20%), phenol (3.60%), steroid (4.80%), terpenoids (1.70%), alkaloids (4.60%), flavonoids (12.20%), and saponin (2.70%). This study recorded tannin (0.24%), alkaloid (3.98%), and flavonoid (2.77%). While phenol and terpenoids were not detected. Again, the findings of Odukoya et al. (2019) recorded finding a total flavonoid of 4.03% from an aqueous extract of sun-dried bitter leaf and 2.14% for a freeze-dried bitter leaf from an ethanolic extract, which also contradicts the concentration of flavonoid (2.77%) in this study. The difference in results could be attributed to various factors, such as the use of different traditional ingredients that are rich in phytochemicals, the processing, preparation, and cooking methods employed in the soup's preparation, and the location from which most of the chief ingredients were sourced (Etsehiwot, 2014; Tiwari & Cummins, 2013; Ukong et al., 2014). It is worth noting that the cooking and food processing methods employed in preparing the soup could have contributed to the reduction in the amount of phytochemicals, especially antinutrients. Atli (2017) previously reported that cooking and food processing methods can reduce the number of phytochemicals present in food.

Conclusion

Nigerian soups, specifically 'Ogbono' and Bitter leaf soups, contain minimal amounts of phytochemicals. Bitter leaf soup contains significantly higher amounts of these phytochemicals compared to ‘Ogbono’ soup. It is also worth noting that the concentration of phytochemicals in the soups generally falls within tolerable limits, except for phenol. Therefore, based on these findings, the moderate consumption of these soups should be encouraged.

Recommendations

Based on the findings of this study, the following are recommended;

1. Ogbono soup should be consumed in moderation due to its high concentration of phenol.
2. Bitterleaf soup contains a substantial amount of phytochemicals that are in moderation, it is therefore suggested that its consumption should be encouraged.
3. Further research should be conducted to identify a processing method that could further reduce the level of phenol in ogbono soups and to understand the impact of processing and preparation methods on the optimal retention of phytochemicals in traditional soups.

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