

Parasite contamination impact on nutritional and phytochemical composition of *Gongronema latifolium*, *Ocimum gratissimum* and *Piper guineense* leaves

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Abstract

Background

Consumption of raw vegetables has been implicated in the transmission of parasites. And as such the cultivation as well as harvesting should be done properly in order to avoid contamination.

Objective

The impact of parasite contamination on the phytochemical and nutritional composition of commonly consumed Nigeria vegetables were determined using *Gongronema latifolium* *Ocimum gratissimum* and *Piper guineense* as case study.

Methods

Both qualitative and quantitative techniques were used to assess the phytochemical and nutritional contents of the plants.

Results

Results showed that parasite contamination had a noticeable impact on the phytochemical contents of the studied vegetables. Alkaloids, flavonoids, saponin, tannin and phenol were slightly present in the contaminated leaves of *G. latifolium* and *O. gratissimum* but were in reasonable amount in non- contaminated. Dry matter was high in both contaminated and non-contaminated (88.72%) and (89.32%) in *G. latifolium*, (89.52%) and (89.72%) in *O. gratissimum* and (89.40%) and (88.40%) in *P. guineense*, Fat was low in both contaminated and non-contaminated (1.70%) and (1.50%) in *G. latifolium*, (1.90%) and (1.70%) in *O. gratissimum*, (1.82%) and (1.67%) in *P. guineense* leaf.

Conclusion

Parasites such as nematodes and protozoa have the capability to consume or degrade proteins in the vegetables which could be the reason for noticeable reduction of proteins in the contaminated samples.. Hence, parasite contamination is capable of affecting the nutritional and phytochemical content of vegetables negatively and undermines the beneficial use of vegetables in amelioration and prevention of diseases.

Introduction

The nutritional benefits of plants are increasingly tremendous in the world today due to their unrestrained roles in human health and constituent active ingredients. Vegetables are fresh edible portions of herbaceous plants which can be eaten raw or cooked and act as important sources of protective foods. Vegetables include leaves, stems, roots, flowers, seeds, fruits, and bulbs. They are regular ingredients in the diet of the average Nigeria and provide appreciable amounts of nutritive minerals. Even though the bulk of their weight is water, leafy vegetables represent a veritable natural pharmacy of minerals, vitamins and phytochemicals. Interestingly, plants produce food in the leaves, but do not store these foods in the leaves. The washing of the vegetables reduces moisture due to water removal from the surface [1]. Parasites like protozoa and nematodes have the ability to introduce mineral-rich material, potentially elevating ash content in contaminated samples [2]. The significance of leafy vegetables has immensely been manifested in the human diet supplying the body with low calorie, substantial amounts of carbohydrates, oil, minerals, vitamins and act as precursors of hormones as well as protein and energy. Moreover, parasites can introduce microbial contaminants that contribute to the breakdown of proteins, further impacting the protein composition [3]. The elucidation of the properties by vegetables are held greatly to the biological active substances possessed by them such as secondary metabolites, high vitamin and mineral contents. They equally help in the

amelioration and prevention of diseases [4]. Alkaloids often act as deterrents to herbivores and pests [5]. Saponins exhibit antimicrobial and pesticidal activities, suggesting their potential effectiveness against parasites contamination. Tannins, known for their astringent properties, could serve as a potential defense mechanism against parasites. Their role in inhibiting microbial growth might indicate their effectiveness against parasitic contamination [6].

Glycosides, compounds often associated with potential therapeutic effects. The presence of glycosides might indicate a specific defense strategy against parasitic threats and absence of glycosides in some plants might suggest alternative mechanisms for coping with parasites. Phenols are associated with a range of health benefits, including their potential to shield against chronic diseases and the aging process [7].

Despite the tremendous benefits of vegetable consumption on human health, there are concerns about the safety of vegetables produced by farmers in rural and some urban settings of developing and underdeveloped countries [8]. In these areas, vegetables are mainly cultivated on untreated soils, using human or animal excreta as a natural fertilizer and untreated wastewater for irrigation [9]. In most cases, after being harvested, these vegetables are consumed raw or processed minimally with less heat or washing to retain the natural taste and preserve heat labile nutrients [10]. This poor hygienic practice promotes the transmission of parasitic eggs to the human host [11]. In other instances, contamination of vegetables may occur during planting as a result of the manure used or after harvesting, with farmers preserving the vegetable through the intermittent sprinkling of unclean water [12]. Vegetables can become contaminated with parasitic pathogens throughout the process of planting to consumption [13]. The extent of contamination depends on so many factors which may include; application of night soil, animal manure and the use of untreated wastewater and water supplies contaminated with sewage as an organic or agricultural fertilizer and for irrigation, coupled with the unhygienic practice of the farmers during harvest, post-harvest handling by vendors, poverty and hygienic conditions of preparation in food service or home. This work examines the effect of contamination on the nutritional and phytochemical content of three commonly consumed vegetable in southeast Nigeria.

Methods

Plant sample collection

About 20g each of fresh leaves of *G. latifolium*, *O. gratissimum*, and *P. guineense* were collected from Federal University of Technology Owerri garden and authenticated by Dr Nwoko of the department of Biology of same University. These vegetables represent some of the

indigenous leafy vegetables that are mainly consumed by the southeastern region in Nigeria.

Sample Preparation

Each sample of *G. latifolium*, *O. gratissimum* and *P. guineense* was divided into two parts. One part of the sample was washed thoroughly and soaked overnight with normal saline to wash away contaminants such as eggs, cysts of parasites and other debrites and termed (non- contaminated sample) while the other part was not washed termed (contaminated sample). Each sample was dried in an oven. After which it was grinded.

8.2g of each grounded sample was taken and soaked in 100 ml of ethanol for 24hrs, after which it was extracted and kept in a room temperature for 48hrs to allow the ethanol to evaporate and a dried sample was obtained. The extracts were weighed and recorded and then used for proximate and phytochemical analysis.

Determination of nutritional composition

The proximate analysis of the leaf extracts of (*G. latifolium*, *O. gratissimum* and *P. guineense*) were carried out to determine the dry matter, ash, moisture content, crude protein, fat, and crude fibre using Association of Official Analytical Chemists (AOAC) method [14].

Phytochemical analysis of the plants extract

The concentrated extracts were subjected to a phytochemical analysis to ascertain the presence of tannin, saponins, phenols, alkaloids, flavonoids, and cardiac glycosides using AOAC methods [14].

Statistical analysis

Laboratory chemical analyses were done in triplicate and the mean value of each chemical parameter was calculated using Microsoft excel. The data was statistically analyzed by using analysis of variance (ANOVA) in Microsoft Excel Tool Pak. Two samples T-test with unequal variances was used to compare mean values and significance was accepted at $P \leq 0.05$ level.

Results

Table 1
Qualitative analysis of Phytochemical components of non-contaminated and contaminated *G. latifolium*, *O. gratissimum*, and *P. guineense*.

Phytochemicals	<i>G.latifolim</i>		<i>O.gratissimum</i>		<i>P.guineense</i>	
	Non-contaminated	Contaminated	Non-contaminated	Contaminated	Non-contaminated	Contaminated
Alkaloids	++	++	++	+++	+	++
Flavonoids	+	+++	+	++	++	++
Saponin	++	++	++	+++	+	++
Tannin	++	+++	+	++	++	++
Phenol	++	++	++	+++	+	++
Glycosides	ND	+	ND	+	ND	+

Table 1: presents the qualitative analysis of various phytochemical components in the leaves of three distinct plants: *G. latifolium*, *O. gratissimum*, and *P. guineense*. The analysis encompasses different conditions, including non-contaminated and contaminated leaves. The presence and intensity of certain phytochemical constituents are categorized using symbols: "+", "++", "+++" and ND; denote slight, medium, heavy presence and not detected respectively.

Table 2
Quantitative Photochemical analysis of *G. latifolium*, *O. gratissimum*, and *P. guineense*.

Vegetables	Alkaloids (mg/100g)	Flavonoids (mg/100g)	Saponin (mg/100g)	Tannin (mg/100g)	Phenol (mg/100g)	Glycosides (mg/100g)
<i>G. latifolium</i>	3.14±0.42 ^a	3.46±0.39 ^a	2.32±0.22 ^a	3.34±0.50	2.09 ±0.35 ^a	0.88±0.30 ^a
<i>O gratissimum</i>	4.09±0.34 ^b	2.08±0.14 ^b	3.90±0.27 ^b	2.02±0.07	2.16±0.23 ^b	0.86±0.22 ^b
<i>P. guineense</i>	2.90±0.03 ^c	2.14±0.14 ^c	2.08±0.11	2.53±0.20	1.76±0.18	1.07±0.28

The table displays the quantitative analysis of different classes of phytochemicals, including alkaloids, flavonoids, saponins, tannins, phenols, and glycosides, in the leaves of these three plants.

Table 3
Proximate analysis of *G. latifolium*, *O. gratissimum*, and *P. guineense*.

Vegetables	MC%	DM%	ASH%	CP%	CF%	FAT%
<i>G. latifolium</i> Leaves	10.49±0.10	88.80±0.31	11.83±0.91	17.85±0.33	11.37±0.15	1.71±0.07
<i>O. gratissimum</i>	10.27±0.13	89.59±0.06	10.16 ±0.27	16.10±0.35	10.14±0.42	1.81±0.05
<i>P. guineense</i>	11.55±0.08	88.76±0.25	12.42±0.04	17.10 ±0.30	12.60±0.17	1.76±0.04

This table presents the results of a proximate analysis conducted on various vegetables, detailing their moisture content (MC%), dry matter (DM%), ash content (ASH%), crude protein (CP%), crude fiber (CF%), and fat content (FAT%). The three vegetables examined are *G. latifolium* leaves, *O. gratissimum*, and *P. guineense*.

Table 4
Quantitative analysis of Proximate contents of non-contaminated and contaminated *G. latifolium*, *O. gratissimum*, and *P. guineense*.

Nutritional contents	<i>G.latifolium</i>		<i>O.gratissimum</i>		<i>P.guineense</i>	
	Non-cont	Conta	Non-cont	Conta	Non-con	conta
MC%	10.66	10.33	10.50	10.04	11.67	11.4
DM%	89.32	88.72	89.72	89.52	88.40	89.40
ASH%	13.41	10.26	10.64	9.69	12.49	12.36
CP%	18.43	17.28	16.71	15.49	17.62	16.58
CF%	11.63	11.12	9.41	10.87	12.31	12.90
FAT%	1.50	1.70	1.70	1.90	1.67	1.82
Non-cont = non-contaminated, conta = contaminated						

This table presents the composition of nutrients in non-contaminated and contaminated samples of *G. latifolium*, *O. gratissimum*, and *P. guineense*. Non- contaminated samples seems to contain more DM (89.32%, 89.72%, 88.40%), Ash (13.41%, 10.64%, 12.49%), CP (18.43%, 16.71% and 17.62%), and FAT (1.50%, 1.70 and 1.67) for *G. latifolium*, *O. gratissimum*, and *P. guineense* respectively.

Discussion

This study revealed intriguing variations in moisture content between non-contaminated and contaminated vegetables. Notably, *G. latifolium* and *O. gratissimum* demonstrate slightly higher moisture content in washed samples, which challenges the conventional understanding that washing reduces moisture due to water removal from the surface [1]. *Piper guineense*, on the other hand, shows elevated moisture content in both non-contaminated and contaminated samples. The presence of parasites could play a role in these moisture content discrepancies [1]. Parasites, including protozoa and nematodes, have the capacity to carry water and contribute to overall moisture content. It is plausible that even the presence of parasites in minute amounts could counteract the anticipated reduction in moisture content associated with washing. In essence, the parasites themselves introduce an additional source of moisture to the samples, potentially causing an offset in the expected decrease. This could explain the higher moisture content observed in washed samples of certain vegetables. The effect of parasitic contamination on moisture content becomes particularly relevant when considering the difficulty in thoroughly removing parasites through standard washing practices. Parasites might adhere more strongly to the vegetable

surfaces, requiring more intensive washing techniques to ensure their removal. This adherence, coupled with the ability of parasites to retain moisture, could be contributing factors to the unexpected moisture content results in washed samples.

The ash content, representing inorganic residue, holds significance in assessing mineral composition. In the context of *G. latifolium*, *O. gratissimum*, and *P. guineense*, This study reveals dynamic variations in ash content between non-contaminated and contaminated samples. Of these vegetables, *P. guineense* exhibits the most distinctive fluctuation in ash content between non-contaminated and contaminated samples. Parasitic contamination introduces a unique facet to ash content variations. Parasites like protozoa and nematodes have the ability to introduce mineral-rich material, potentially elevating ash content in contaminated samples. Their presence amplifies the complexity of assessing ash content accurately, particularly in *Piper guineense*, which appears to be more susceptible to the influence of parasites. While washing is usually thought to lower ash content by removing extraneous material, this assumption may not be accurate due to parasitic contamination potentially adding its own contribution that offsets the effects of washing [2]. Crude protein content serves as a key indicator of nutritional quality, encompassing the essential amino acids vital for human health [3]. Among these vegetables as presented, *P. guineense* appears to be the most variable in terms of crude protein content between non-contaminated and contaminated samples. The presence of parasites such as protozoa and nematodes introduced an intricate layer of influence on this parameter. These parasites have the capability to consume or degrade proteins present in the vegetables, leading to a noticeable reduction in crude protein content in contaminated samples. Moreover, parasites can introduce microbial contaminants that contribute to the breakdown of proteins, further impacting the protein composition [3]. This highlights the significance of recognizing the presence of parasites, particularly in *P. guineense*, when analyzing and interpreting crude protein content.

Crude fiber content is a critical parameter reflecting the indigestible components of vegetables. Examining *G. latifolium*, *O. gratissimum*, and *P. guineense* by comparing their crude fiber content between non-contaminated and contaminated forms, *P. guineense* displays significant variations in crude fiber content between non-contaminated and contaminated samples. The presence of parasites introduced a fascinating dimension to crude fiber content changes. Parasites, including protozoa and nematodes, could potentially contribute to the observed variations. Their activity may lead to the breakdown of fiber constituents or, conversely, the introduction of additional fiber-rich material. This dynamic underscores the intricate interplay between parasitic contamination and crude fiber assessment, particularly pronounced in *P. guineense*.

A comprehensive qualitative analysis of the phytochemical constituents present in the leaves of three distinct plant species: *G. latifolium*, *O. gratissimum*, and *P. guineense*. The presence and intensity of specific phytochemical components are categorized using standardized symbols to indicate their relative abundance: "+", "++", and "+++" denote slight, medium, and heavy presence, respectively, while "ND" (Not Detected) signifies the absence of a particular constituent.

Alkaloids, which encompass a diverse and intricate collection of cyclic compounds containing nitrogen, consistently impart a bitter taste. Alkaloids have been linked to a wide spectrum of pharmacological roles, including but not limited to antimalarial, anticancer, cholinomimetic, vasodilatory, antiarrhythmic, antibacterial, antihyperglycemic, psychotropic, and stimulant properties [4]. In *G. latifolium*, *O. gratissimum*, and *P. guineense*, alkaloids are denoted as "++" in contaminated samples, indicating a medium presence. In non-contaminated samples, *G. latifolium* and *P. guineense* exhibited a medium presence, while *O. gratissimum* displays a heavy presence of alkaloids ("+++"). The presence of alkaloids in varying intensities across all three plants suggests their potential role in defense mechanisms against parasites. Alkaloids often act as deterrents to herbivores and pests, and their increased presence in contaminated *O. gratissimum* might indicate a response to parasitic threats. Parasites could potentially influence alkaloid synthesis, leading to alterations in their concentration. Additionally, the observed differences could arise from the plant's attempts to cope with the presence of parasites through biochemical changes [5].

Comparing saponin content between non-contaminated and contaminated states provides valuable insights into potential effects of parasitic contamination. In the contaminated state, *G. latifolium* and *O. gratissimum* display a medium presence ("++"), while *P. guineense* demonstrates a heavy presence ("+++"). After washing, *G. latifolium* and *O. gratissimum* maintained

a medium presence, while *P. guineense* retained its heavy presence of saponins. The presence of saponins across all three plants, with varying intensities, implies a potential role in defense mechanisms against parasites. Saponins often exhibit antimicrobial and pesticidal activities, suggesting their potential effectiveness against parasites. The maintenance of saponin presence after washing might indicate their stable contribution to the plants' defense strategies. Parasitic contamination might influence the synthesis of saponins, resulting in variations in intensity between washed and unwashed states. Consequently, the variations in saponin content might be linked to the plants' responses to the presence of parasites and their attempts to deter potential threats [5].

Contaminated *G. latifolium* and *O. gratissimum* show a slight presence ("+"), whereas *P. guineense* displays a medium presence ("++"). Washing has minimal effect on *G. latifolium* and *O. gratissimum*, maintaining their slight presence of tannins. However, non-contaminated *P. guineense* exhibits an increased intensity of tannins ("+++"). These variations highlight the potential influence of washing on tannin content in these plants. Tannins, known for their astringent properties, could serve as a potential defense mechanism against parasites. Their role in inhibiting microbial growth might indicate their effectiveness against parasitic contamination. The variation in tannin content between non-contaminated and contaminated *P. guineense* could suggest that parasites influence tannin synthesis. Additionally, the presence of tannins across the contaminated samples might point toward their role in plant protection against parasitic threats [6]. Glycosides, compounds often associated with potential therapeutic effects. The presence of glycosides in *O. gratissimum* and their absence in *G. latifolium* and *P. guineense* highlights potential differences in their chemical profiles. The presence of glycosides might indicate a specific defense strategy against parasitic threats in *O. gratissimum*. The absence of glycosides in the other two plants might suggest alternative mechanisms for coping with parasites.

Phenols are associated with a range of health benefits, including their potential to shield against chronic diseases and the aging process [7]. Phenols, compounds associated with various biological activities, are present across all three plants. Contaminated *G. latifolium* and *O. gratissimum* exhibit a medium presence ("++"), while *P. guineense* displays a heavy presence ("+++"). After washing, *G. latifolium* maintains a medium presence, *O. gratissimum* shows a heavy presence, and *P. guineense* continues to exhibit a heavy presence of phenols. This suggests that washing may not significantly alter the presence of phenols in these plants. The presence of phenols, often associated with antioxidant and antimicrobial properties, suggests a potential role in combatting parasitic contamination. Parasites might influence phenol synthesis, leading to variations in their presence and intensity. The consistent presence of phenols after washing could indicate their importance in the plants' responses to both biotic and abiotic stressors.

Conclusion

In conclusion, parasite contamination is capable of affecting the nutritional as well as the phytochemical composition of vegetables and disrupt the nutritional and medicinal benefits. In order to get the best benefits from vegetables, it should be kept away from parasitic contamination starting from the time of planting to consumption.

Abbreviations

MC
Moisture content
DM
Dry matter
ASH
Ash content
CP
Crude protein
CF
Crude fibre

ND
Not Detected
Km
Kilometer
%
Percentage
Fig
Figure
g
Gram
mg
Milligram
hrs
Hours
AOAC
Association of Official Analytical Chemists
ANOVA
Analysis of variance.

Declarations

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We acknowledge all the authors for their immense contribution towards this work.

Authors contributions

The work was conducted in collaboration among all authors. Author SNN designed the study, wrote the first draft of the manuscript. Authors ICO, MCI, and CA managed the analysis of the study. Author MON did the statistical analysis. Authors MCN, VCU, BCU and MOU managed the literature searches. All the authors read through the manuscript and approve the publication.

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Data availability statement

All data generated or analysed during this study are included in this published article.

Conflict of interest

All the authors declare that there is no conflict of interest and agreed to the publication of the work.

Ethics and Guidelines

Ethical approval was given by **Micheal Okpara University of Agriculture, College of Natural Sciences Research Ethical Committee**. This research work followed the **Guidelines for Research Ethics in Science and Technology**.

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Figures



i



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Figure 1

Shows the images of the leafy vegetables used in the study (i) *P. guineense* commonly known as uziza, (ii) *Gongronema latifolium* commonly known as utiza and (iii) *ocimum gratissimum* commonly known as scent leaf.