VER
Vascular & Endovascular Review

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 (Gongronema latifolium. Ocimum gratissimum and Piper guineense) were determined to ascertain the extent contamination can influence beneficial potential of these vegetables which are sometimes consumed raw. **Methods**: Both qualitative and quantitative techniques were used to assess the phytochemical and nutritional composition of these vegetables both when contaminted or not. The parasites present in the contaminated samples were also determined through floatation method and examining under the microscope. **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. Parasites such as Entamoeba histolytica, Giardia lamblia, Cryptosporidium spp, Strongyloides spp, Toxocara spp. were identified in all the contaminated vegetables. **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.

How to Cite: Nmezi Stella, (2026) Parasite contamination impact on nutritional and phytochemical composition of Gongronema latifolium, Ocimum gratissimum and Piper guineense leaves, Vascular and Endovascular Review, Vol.9, No.1, 280-286

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 pesticide 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 right 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.

Table 1: Phytochemicals, and Medical significance of *G. latifolium, O . gratissimum, and P. guineense*

Name of Vegetable	Phytochemicals	Medicinal
G. latifolium		**it is, apart from its culinary uses, employed in traditional medicine [16]. The bark contains some quantity of latex and though it has been viewed with potential interest for its rubber, it has apparently never been exploited.**
O . gratissimum		**The plant is economically important for its essential oil and folklore medicinal use and insecticidal properties [11]. The essential oil of the plant has been widely used in food,cosmetic,pharmaceutical and soap industries**
P. guineense	**Ethyl iso-allocholate, i-propyl l9,12octadecenadienoate, 1-Heptatriacotanol, βStigmasterol -sitosterol, Crinamidine,3-oxo- , Vitamin E, Androstan-3-one,17methoxy-3methoxime,(5a,17 β)- ,Bis-(3,4-dimethoxy phenyl)hydroxyacetic acid methyl ester, Linoleic acid ethyl ester,2-[4-methyl-6(2,6,6trimethylhex1-enyl)hexa-1,3,5trienyl]cyclohex-1carboxaldehyde**	***Piper guineense* is pharmacological important for its medicinal, cosmetic and insecticidal uses [14]. It provides oil used as aromatics in the drink industry [15].**

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 botanist from the department of Biology of same University. These vegetables represent some of the indigenous leafy vegetables that are mainly consumed raw 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 sugar and salt solution to wash away contaminants and float eggs and cysts of parasites and other debris and termed (non- contaminated sample) . The solution was centrifuged to concentrate the parasites and examined under the microscope, 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 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 Park. 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	*G. latifolium* NC	C	*O .gratissimum* NC	C	*P. guineense* NC	C
Alkaloids	++	++	++	+++	+	++
Flavonoids	+	+++	+	++	++	++
Saponnins	++	++	++	+++	+	++
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.

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)
G. latifolium	3.14 ± 0.42^a	3.46 ± 0.39^a	2.32 ± 0.22^a	3.34 0.50	$2.0 \pm .35^a$	0.88 ± 0.30^a
O gr atissimum	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
P. Guineense	2.90 ± 0.03^c	$2.1 \pm 4\ 0.14^c$	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, flavonoid, saponins, tannins, phenols, and glycosides, in the leaves of these three plants

Proximate analysis of *G. latifolium, O . gr atissimum, and P. guineense*

Vegetables	MC%	DM%	ASH%	CP%	CF%	FAT%
G.latifolium	**10.4± 0.10**	**88.8± 0.31**	**11.8± 0.91**	**17.8± 0.33**	**11.3± 0.15**	**1.7± 0.07**
O . gratissimum	**10.2± 0.13**	**89.5± 0.06**	**10.1± 0.27**	**16.1± 0.35**	**10.1± 0.42**	**1.8± 0.05**
P. Guineense	**11.5± 0.08**	**88.7±0.25**	**12.4± 0.04**	**17.1± 0.30**	**12.6± 0.17**	**1.7±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 ber (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	***G.latifolium*** NC	C	***O .gr atissim*** NC	C	***P.guineense*** NC	C
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

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.

Table 5: Vegetables and parasites detected

Vegetable type	Parasite detected
G. latifolium	*Entamoeba histolytica, Giardia lamblia, Cryptosporidium spp, Strongyloides spp, Ascaris lumbricoides, Enterobius vermicularis, Trichuris trichiura, Toxocara spp.*
O .gratissimum	*Entamoeba histolytica, Giardia lamblia Cryptosporidium spp Strogyloides spp, Toxocara spp, Hymenolepis nana, H. diminuta, Cystoisospora belli*
P. Guineense	*Entamoeba histolytica, Giardia lambliaCryptosporidium spp, Strongyloides spp, Ascaris lumbricoides, Fasciola hepatica, Hookworms, Toxocara spp, Hymenolepis nana, Toxoplasma gondii*

DISCUSSION

This study revealed intriguing variations in moisture content between non-contaminated and contaminated vegetables. Notably, *G. latifolium and O . gr atissimum* demonstrated 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* exhibited 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 implies 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* appeared 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 highlighted 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 representing 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* displayed 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 could 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 phytochemical constituents present in the leaves of these distinct plant *species G. latifolium, O . gratissimum, and P. guineense*: . The presence and intensity of specific phytochemical components are categorized using standardized symbols to indicated their relative abundance: "+", "++", and "+++" denote slight, medium, and heavy presence, respectively, while "ND" (Not Detected) signified 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, vasodilation, anti-arrhythmic, antibacterial, hyperglycaemia, psychotropic, and stimulant properties [4]. In *G. latifolium, O . gr atissimum, 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* displayed a heavy presence of alkaloids ("+++"). The presence of alkaloids in varying intensities across all three plants suggested 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* displayed a medium presence ("++"), while *P. guineense* demonstrated 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, implied a potential role in defense mechanisms against parasites. Saponins often exhibit antimicrobial and pesticide 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* showed a slight presence ("+"), whereas *P. guineense* displayed a medium presence ("++"). Washing has minimal effect on *G. latifolium and O . gr atissimum* , maintaining their slight presence of tannins. However, non-contaminated *P. guineense* exhibited an increased intensity of tannins ("+++"). These variations highlighted 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 influenced 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 . gr atissimum* and their absence *in G. latifolium and P. guineense* highlighted 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* exhibited a medium presence ("++"), while *P. guineense* displayed a heavy presence ("+++"). After washing, *G. latifolium* maintains a medium presence, O . gratissimum showed a heavy presence, and *P. guineense* continues to exhibit a heavy presence of phenols. This suggested 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 combating 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
g Gram
mg Milligram
hrs Hours
ANOVA Analysis of variance.

Declarations

Acknowledgement
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. Author MCN managed the analysis of the study. Author JL did the statistical analysis. Authors VCU and OEO managed the literature searches. JCO did the proofreading. All the authors read through the manuscript and approve the publication.

Funding
No funding received

Data availability statement
All data generated or analyzed 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.

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Figures

Fig i

Fig ii

fig iii

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) O*cimum gratissimum* commonly known as scent leaf.