

REVISED VERSION OF THE MANUSCRIPT

Parasitic contamination of fresh bitter leaf vegetables - Onugbu (*Vernonia amygdalina*): a case study of farms in Nimo, Njikoka Local Government Area, Anambra State, Nigeria

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Abstract

Aim: Vegetables form a major component of the human diet; however, some agricultural practices may put consumers at risk of parasitic infections. There is high consumption of bitter leaf (Onugbu) in the rural part of Nigeria. They are eaten raw as medicinal herbs by some people. The culture of inadequate or poor washing of vegetables could pose significant and major health threats to the consumers. The aim of the study was to identify some of the parasites that may be attached to the locally consumed edible vegetable *Vernonia amygdalina* called Onugbu amongst Igbo speaking dialect in south eastern Nigeria.

Study Design: This study is a survey that evaluated the parasitic contamination of bitter leaf grown in selected farms in Nimo in Njikoka Local Government Area of Anambra State, Nigeria.

Duration: The study lasted for six months.

Methodology: *V. amygdalina* leaf samples were bought directly from the farmers at 300 Naira per rap. The examination of sample was carried out using sedimentation method. Samples weighing 50g were gently washed in normal saline water. The water used in washing was filtered and centrifuged at 300g to concentrate the parasitic stages and also to avoid damages. The sediments obtained were transferred unto labelled clean slides and examined under the light microscope at x 40 objective lens.

Results: Parasites were detected in all samples collected from the farm. The commonest parasite was *Entamoeba histolytica* (cyst 45.5%); other parasites seen are *Entamoeba coli* (cyst 13.6%), *B. coli* (trophozoite 27.3%), *Ascaris lumbricoides* (ova 9.1%) and *Strongyloides stercoralis* (larva 4. 6%).

Conclusion and recommendations: The results provide evidence of contamination of bitter leaves from farms in Nimo in Njikoka Local Government Area of Anambra State, Nigeria with parasites of public health importance. To avoid infection, humans should wash the leaves of this vegetable with running water or salt and water before it is eating raw.

Key words: Parasite, contamination, vegetable, *Vernonia amygdalina*, Nimo, Anambra State and Nigeria.

Introduction

The consumption of vegetables has increased in recent years because of their nutritional importance, health benefits and constituent of a balance (healthy) diet [1]. A diet rich in vegetables and fruits can lower blood pressure, reduce the risk of heart disease and stroke, prevent some types of cancer, lower risk of eye and digestive problems, and have a positive effect upon blood sugar, which can help keep appetite in check. Eating non-starchy vegetables and fruits like apples, pears, and green leafy vegetables may even promote weight loss [2]. Vegetables are also vital sources of energy that are depended upon by all levels of human as food supplements or nutrients [3, 4]. *Vernonia amygdalina* commonly called bitter leaf or Onugbu (Onugbu is the local name for *Vernonia amygdalina* amongst Igbo speaking dialect in south-eastern Nigeria) is the most widely cultivated species of the genus *Vernonia* which has about 1,000 species of shrubs. *V. amygdalina*, a member of the family Asteraceae, is a widely used vegetable that can grow in different parts of the world [5]. *V. amygdalina* is frequently found in gardens, and can adapt to a variety of climatic conditions, unlike other plants. *V. amygdalina* occurs naturally along rivers and lakes, in woodland and grassland up to 2800 m, in regions where the mean annual rainfall is 750-2000mm [6]. [7]. It requires full sunlight and grows in a humid environment. It can also grow on all soil types but *V. amygdalina* can be commonly found along drainages and in natural forests or at home and commercial farms is best planted on humus-rich soils [6]. Although is popularly used for food, it has also, been traditionally used for its medicinal purposes [8].

Cooked leaves of *V. amygdalina* are a staple vegetable in soups and stews of various cultures throughout Nigeria. It is known as Ewuro in Yoruba, Etidot (Ibibio), Ityuna (Tiv), Oriwo (Edo) and Chusar-doki (Hausa) [9]. The leaves are green in colouration and have a bitter taste [10]. Their traditional use is not only limited to humans alone as it added to horse feed to provide a strengthening or fattening tonic known as ‘Chusan Dokin’ in Northern Nigeria [7].

According to WHO, some parasites, such as fish-borne trematodes, are only transmitted through food. Others, for example tapeworms like *Echinococcus spp.*, or *Taenia solium*, may infect people through food or direct contact with animals. Other parasites, such as *Ascaris*, *Cryptosporidium*, *Entamoeba histolytica* or *Giardia*, enter the food chain via water or soil and can contaminate fresh produce [12]. Parasitic infections are mostly spread by human activities which may include: Poor personal hygiene and consumption of unwashed or poorly washed vegetables and fruits [13]. Intestinal parasites that are common in raw vegetables include protozoa, nematodes, cestodes, and trematodes [14]. Studies have shown that *Ascaris lumbricoides*, *Cryptosporidium spp.*, *Entamoeba histolytica*, *Enterobius vermicularis*, *Fasciola spp.*, *Giardia intestinalis*, hookworms, *Hymenolepis spp.*, *Taenia spp.*, *Trichuris trichiura* and *Toxocara spp.* can infect humans who consume contaminated, uncooked or improperly washed vegetables and fruits [15]. There is high consumption of vegetables especially bitter leaf (Onugbu) in the rural part of Nigeria. This may be associated with its health benefites. *Vernonia amygdalina* is rich in nutrients and phytochemicals and this invariable account for its positive influence on health. Compounds associated with its anticancer effects, antioxidant properties, antimalarial properties, anti-inflammatory properties, antimicrobial properties and hypolipidaemic effects have been identified but most mechanisms of action have not been elucidated in humans [16]. Research has also showed that the metabolites in the leaf have specifically been efficacious against parasites especially worms. The mechanisms of activities include paralysis of worm, interference with energy generation and impairment with

nutrient absorption, motility, and reproduction [6]. *V. amygdalina* is used in traditional herbal medicine. This include alleviating malnutrition that may be due to micronutrient deficiencies, useful in the treatments for diabetes, lowers cholesterol and is used in treatment of infections [17].

They are eaten raw or mashed to get the juice used in treating abdominal difficulties such as diarrhea, stomach upset, and gastrointestinal tract diseases like dysentery and related other issues. They are also eaten after de-bettering through normal squeeze-washing. Despite the health benefits of the leafy vegetable, little or no information exists on the possibility of it been a source of infection to the consumer through contamination. The culture of inadequate or poor washing of vegetables could pose significant and major health threats to the consumers who at times eat this vegetable raw. This study therefore, was conducted to determine the level of parasitic contamination of this plant *V. Amygdalina* in Nimo, Njikoka Local Government Area in Anambra State, Nigeria.

2. Materials and Methods

Study Area: This study was carried out in Nimo Njikoka Local Government Area of Anambra State, Nigeria. Nimo community is located in Southern Nigeria; the people in the state are predominantly Igbo. It is located between Latitude 6° and 9' 25" N and Longitude 6° and 59' 21" E. The monsoon wind from the Atlantic ocean creates seven months of heavy tropical rain which occur between April to October which is followed by five-month of dryness [16].

Study Design: The study is an experiment carried out using the sedimentation method.

Sampling Areas: The Bitter leaf was collected from farms in Nimo Njikoka Local Government of Anambra State, Nigeria. These farms were considered because of their booming commercial sales of Onugu leaf to different markets in south-eastern Nigeria.

Samples Collection: Samples were collected randomly from the three different farms. Each sample was bought directly from the farmers within that area at 300 Naira per parcel, were placed in a separate sterile polythene bag, labelled with a unique number and transported taken to Zoology laboratory in Nnamdi Azikiwe University, Awka for analysis.

Examination of Sample for Parasites: The examination of the sample was carried out using the sedimentation method. Approximately 50g of the bitter leaf were soaked for 15 minutes separately in round bottom plastic containers with 150ml of normal saline (0.90% NaCl) to dislodge the parasitic stages (ova, larvae and cyst) of helminths and protozoan parasites that may have contaminated the vegetables. The samples were gently washed out into a labelled separate plastic container with normal saline for 15 minutes. The solutions were collected into different flat bottom conical flask labelled with the name of each of the three farms and where allowed to settle for 10 minutes. The supernatant was discarded leaving about 20ml of the washed samples at the bottom. The deposited mixtures were sieved through a fine guaze and then transferred to a centrifuge tube and centrifuged at 3000 rpm (1,200g) for five minutes [17]. After centrifugation, the supernatant was discarded while the sediment obtained was transferred onto a labelled clean slide and examined under the light microscope under 10x and 40x objectives for identification. Parasites seen were recorded.

Statistical Analysis: Data entry and analysis were carried out. Frequency was expressed in percentage; prevalence was analyzed with ANOVA using SPSS version 25. Mean intensity was calculated and analyzed with ANOVA.

3. Results

Isolation and identification of parasites

The study showed that all the bitter leaves gotten from these farms were contaminated with one or two parasite species. Five parasites: *Entamoeba histolytica*, *Balantidium coli*, *Entamoeba coli*, *Strongyloides stercoralis* and *Ascaris lumbricoides* were recovered from the vegetables examined in the three farms. *Entamoeba histolytica*, *Balantidium coli* were isolated in farm A, *Entamoeba histolytica*, *Entamoeba coli* were isolated from farm B and *Strongyloides stercoralis* and *Ascaris lumbricoides* from farm C. Among parasites recovered, *Entamoeba histolytica* was the most commonly found parasite in the study (10) followed by *B. coli* (6 parasites), *E. coli* (3 parasite), *A. lumbricoides* (2 parasite) and the least is *S. stercoralis* (1 parasite). It was observed that Farm (A) had the most affected samples (5 cysts and 6 trophozoites) followed by farm B (8 cysts) and farm C has 2 ova and a larva (Tables 1 and 2).

Table 1: Parasites isolated and identified from *V. amygdalina* soled in Nimo farms in Njikoka Local Government Area, Anambra State, Nigeria

Farm	Weight of bitter leaf	Types of parasite	No. of parasite seen	Stages of parasite
A	50	<i>Entamoeba histolytica</i> ,	5	Cyst
		<i>Balantidium. Coli</i>	6	Trophozoite
B	50	<i>Entamoeba histolytica</i>	5	Cyst
		<i>Entamoeba coli</i>	3	Cyst
C	50	<i>Ascaris lumbricoides</i>	2	Ova
		<i>Strongyloides stercoralis</i>	1	Larva
Total			22	

E. histolytica had the highest number of occurrence in the three farms (45.5%) followed by *B. coli* (27.3%), *E. coli* (13.6%), *A. lumbricoides* (9.1%) while the occurrence of *S. stercoralis* (4.6%) was very scanty, no bitter leaf examined in farm (C) was contaminated with *E. coli*, *E. histolytica*, or *B. coli* (Table2).

Table 2: Distribution of parasite species found in *V. amygdalina* farms in Nimo, Njikoka Local Government Area, Anambra State, Nigeria

Farm	<i>E. coli</i>	<i>E. histolytica</i>	<i>B. coli</i>	<i>A. lumbricoides</i>	<i>S. stercoralis</i>	Total parasites
A	0	5	6	0	0	11
B	3	5	0	0	0	8
C	0	0	0	2	1	3
Total parasites	3	10	6	2	1	22
% occurrence	13.6	45.5	27.3	9.1	4.6	100

In table 3 *E. histolytica* had the highest prevalence and mean intensity of parasites (33.30% 3.33±1.67) while other isolated parasites had the same prevalence of 16.70% and different mean intensity of parasites (*Balantidium coli* 2.00±2.00, *Ascaris lumbricoides* 0.67±0.67, *Strongyloides*

stercolaris 1.67±0.67 and *Entamoeba coli* 0.33±0.33). However, there was no significant association between parasite species and prevalence (P= 0.893).

Table 3: Prevalence and mean intensity of parasites found on fresh bitter leaf vegetables (*V. amygdalina*) sold in farms in Nimo, Njikoka Local Government Area, Anambra State, Nigeria

Parasite specie	No. of bitter leaf farms examined	No of infected Farms	Total parasite found	Prevalence (%)	Mean intensity
<i>Entamoeba histolytica</i>	3	2	10	33.30	3.33±1.67 ^{a*}
<i>Balantidium coli</i>	3	1	6	16.70	2.00±2.00 ^{a*}
<i>Ascaris lumbricoides</i>	3	1	2	16.70	0.67±0.67 ^{a*}
<i>Strongyloides stercolaris</i>	3	1	1	16.70	1.67±0.67 ^{a*}
<i>Entamoeba coli</i>	3	1	3	16.70	0.33±0.33 ^{a*}

*Means in columns with similar superscripts are not significantly different from each other

4. Discussion

The habit of eating raw unwashed and undercooked vegetables (*V. amygdalina*) plays a critical role in the transmission of parasitic diseases [17, 18 & 15]. This work on parasitic contamination of bitter leaf in farms in Nimo community, Anambra State in Nigeria revealed that bitter leaf sold in various markets around the community were contaminated with protozoan and helminth parasites. Intestinal parasites have become a major public / national health concern in the developing countries of the world, especially countries like Nigeria [19, 20]. Like many other tropical countries, the burden of intestinal parasites in Nigeria has been greatly encouraged by the favourable climatic conditions, lack of awareness on the danger and need for prevention and control of parasitic diseases, and the poor farming practice, the poor sanitary practices amidst farmers and vendors who cultivate and sell these vegetables [21]. This study revealed that *V. Amygdalina* gotten from farms in Nimo were contaminated by various levels of helminth and protozoan parasites of human medical health importance. These results are in agreement with the results of several studies on leafy edible vegetables across Nigeria [22, 23 & 24]. This may be attributed to the fact that many farmers use organic manure as fertilizer. The use of organic manures (animal dung) as fertilizer may be one of the factors aiding the development of parasites on soil and subsequent contamination of vegetables [25]. Various prevalence rates of the contamination was recorded in this study; this may also be due to the different epidemiological factors such as climatic conditions of an area, type of water and fertilizer used for growing this bitter leaf and contamination after harvest [26]. Also the season of the year when this study was conducted can influence the prevalence of contamination. The study was conducted in dry season of the months October to April. According to Yusof (2017) there was a high diversity in the type of parasites observed during the dry season compared to the monsoon season. Also, animal manure is widely used as fertilizer to improve the soil fertility. Some of these parasites may have been attached to the vegetables from the manure used or from the contaminated water used in watering the farms especially during the dry season. According to Uyttendaele *et al.* (2015) numerous studies have reported the extended survival of those enteric pathogens in manure and manure-amended soil, which can subsequently contaminate fresh produce growing in the field.

vegetables were predominantly contaminated by the cyst of *E. histolytic* in this study. This result is in agreement with the study of [27]. Sometimes when bitter leaves are processed the water which is gotten could lead to infection and disease especially when humans drink them raw [28] to cure sickness. The occurrence of parasites on vegetables is a potential threat to public health, despite the numerous efforts and resources channelled towards combating food-borne related infections.

Conclusion

This study showed that vegetables (Bitter leaf) gotten in the selected farms in Nimo, Njikoka Local Government Area of Anambra State, Nigeria were contaminated with medically important parasites which are sources of disease transmission. These findings have shown that consumers of these vegetables stand a high risk of being infected with intestinal parasites. Therefore, improved hygiene among the farmers, consumers, and vendors will be paramount in reducing the burden of intestinal parasite infection

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