

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

Abstract

Aim: Vegetables form a major component of the human diet; however, some agricultural practices may put consumers at risk of parasitic infections. 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]. *V. amygdalina* can be commonly found along drainages and in natural forests or at home and commercial farms [7]. It requires full sunlight and grows in a humid environment. It can also grow on all soil types but 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 [11].

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*, *Crysporidium spp.*, *Entamoeba histolytica*, *Enterobus vemicularis*, *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. 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 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 separate plastic container and the saline was collected into different flat bottom conical flask labelled with the name of each of the three farms. The supernatant was discarded leaving about 20ml at the bottom. The deposited mixture was 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. Among parasites recovered, *Entamoeba histolytica* was the most commonly found parasite in the study (10). It was observed that Farm (A) had the most affected samples (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>		Ova
		<i>Strongyloides stercoralis</i>	2	
			1	Larva
Total			22	

E. histolytica had the highest number of occurrence in the three farms (45.5%) (Table 2). 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

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*}

There was no significant association between parasite species and prevalence (P= 0.893)

*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]. The 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 Anamabra 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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