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Intestinal Parasites Load and Microscopy of the Leaves of *Telfairia occidentalis* Hook. F (Cucurbitaceae) in Niger Delta Region of Nigeria

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Abstract: *Telfairia occidentalis* Hook. F. (Cucurbitaceae) is well known in West Africa as a staple vegetable. It is used among various tribes of Nigeria to manage various conditions such as sexual impotence, maintenance of prostate gland, urinary and digestive disorders and it is also used as immune-stimulant and vermifuge. Vegetables have been known to harbour parasites. The aim of this work is to determine the level, type of intestinal parasites on the leaves and the diagnostic characters of the plant for proper identification. Fifty-one samples of leaves cultivated in flooded and unflooded areas in Amassoma were collected between April and August 2013 and the parasites isolated by standard microscopic methods. The microscopy of the leaves was carried out. The most prevalent parasite was *Ascaris lumbricoides* (44%) followed by *Trichuris trichiura* (17%), *Entamoeba coli* (12%), Hookworm (7.%) while the least was *Strongyloides stercoralis* (2%). The samples from flood affected areas showed a higher prevalence rate of 20% than those from flood free areas for *Ascaris lumbricoides*. All the parasites except hookworm were present in the samples sold in the market. Anomocytic stomata were present in the lower surface of leaves other diagnostic characters present in the leaves include glandular, multicelled glandular trichomes, covering trichomes, prsims of calcium oxalate crystals and starch granules. The study shows that *Ascaris lumbricoides* is the major intestinal contaminant of *Telfairia occidentalis* vegetable consumed in Amassoma, Nigeria.

Key words: Microscopy · Instestinal Parasites · *Ascaris Lumbricoides* · *Telfairia Occidentalis* · Vegetable

INTRODUCTION

Telfairia occidentalis Hook. F. (Cucurbitaceae) commonly known as fluted pumpkin originates from West Africa and occurs frequently in Benin, Nigeria and Cameroun. It is rare in Uganda and absent in East Africa [1-8]. It has been suggested that it originated from South Eastern Nigeria and distributed by the Igbos. The wild plant has been harvested into extinction and is now replaced by cultivated forms [9]. It is a staple vegetable grown in Nigeria and popular for its nutritional, medicinal and industrial values [10-12]. It is known by several common names: Ugu (Igbo), Iroko or Apiroko (Yoruba), Ubong (Efik), Umee (Urhobo), Umeke (Edo) [13-14]. The seeds are also nutritious and rich in oil which may be used for cooking and soap making [8]. It is a dioecious, perennial and drought tolerant climber [6, 15-19], planted with stakes of various types including bamboo. The leaf's juice has been used to treat sexual impotence, urinary and digestive disorders, maintenance of prostate gland and as immune-stimulant and vermifuge [20]. It is employed for the treatment of anaemia, chronic fatigue and diabetes [21-22]. It can be eaten cooked and constitutes the main vegetable of the Nigerian delicacy referred to as "Edikang ikong" soup [13, 18, 23-24]. Although the raw leaf may not be chewed directly, it is usually squeezed and used as a short term blood tonic [25]. It has been reported to have hepatoprotective activity [26], hypolipidaemic [27] effect in rats fed with cholesterol rich diet [4], erythropoietic effect [28] and hypoglycaemic effect [29-30]. It is very rich

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in minerals especially iron [31], phenol and amino acids [18, 31-34]. The root and the leaves contain alkaloids and saponins [35-37]. The local preparation of the plant for medicinal use entails squeezing the juice and for food it is cooked after rinsing; therefore, the intestinal parasitic load of the leaves was investigated as part of determining the safety profile of the plant. Macroscopical and microscopical characteristics of the leaves were carried out to ensure proper identification and identity of adulteration.

MATERIALS AND METHODS

Description of Study Area: Amassoma is the headquarter of Ogboin clan as well as Ogboin-North Rural Development Authority in the Southern Ijaw Local Government Area of Bayelsa State (Fig. 1 & 2). It is the host community to Niger Delta University, Wilberforce Island, Bayelsa. It is located about 40 km to the South of Yenagoa; the State capital. It is on an altitude of 512 above sea level, bound in the North by River Nun, West by Otuan, East by Toru Ebeni and the South by Ogobiri [38]. It is the biggest town in Southern Ijaw Local Government Area whose headquarter is in Oporoma in the north area. Amassoma has a coastline of approximately 60 km on the Bight of Bonny. It has an area of 2,682 km^2 and a population of 319,413 at the 2006 Census [39].

Study Design / Sample Collection: *Telfairia occidentalis* (Plate 1) was identified and authenticated by Mr. Oladele, of the Department of Forestry and Wild Life Management, University of PortHacourt, Nigeria. A herbarium specimen was made and a voucher specimen (NDUP 089) has been deposited at the herbarium, Department of Pharmacognosy and Herbal Medicine, Niger Delta University, Nigeria. Forty-one leaf samples were collected from different cultivated plants including flood affected areas in Amassoma. Ten additional samples were obtained from the main market of the town. The leaves were collected between April and August 2013.

Sample Analysis

Microscopical Examination for Intestinal Parasites: Sample weighing 50 g of each batch of vegetable was examined for intestinal parasite profile as described by Nyarango *et al*. [40]. The sample was washed in distilled water and the suspension was strained through a sterile sieve to remove undesirable materials [40]. The filtrate was centrifuged at 3000 rpm for 5 minutes and the supernatant discarded while the deposit was placed on a glass slide and a cover slip was placed on it, it was examined under a light microscope using 10x and 40x objective to identify the cysts and eggs [41]. Different parasite eggs, larvae or cysts present in the samples were counted.

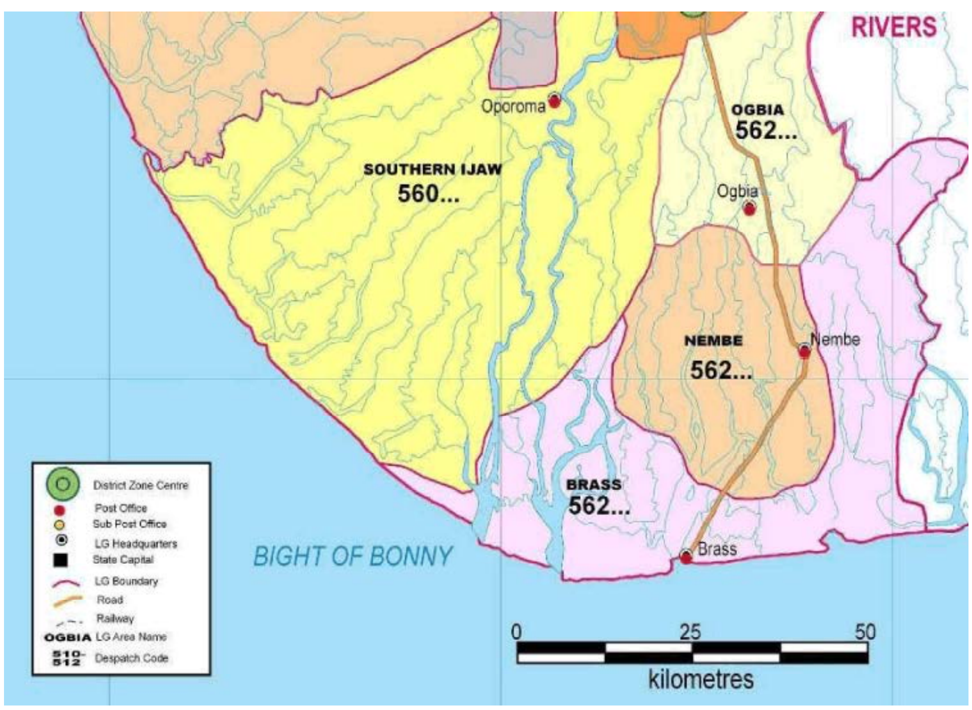


Fig. 1: Map of Bayelsa State showing Southern Ijaw Local Government Area

Fig. 2: Map of Southern Ijaw showing location of Amassoma

Plate 1: Photograph of *Telfairia occidentalis*

Data Analysis: Parasitological indices were analysed according to standard method [42]. It is described as follows:

$$\text{Prevalence of Parasites} = \frac{\text{Number of samples infested}}{\text{Number of samples examined}} \times 100$$

$$\text{Intensity of Parasites} = \frac{\text{Number of parasites found}}{\text{Number of samples infested}} \times 100$$

$$\text{Abundance of Parasites} = \frac{\text{Number of parasites found}}{\text{Number of samples examined}} \times 100$$

Data was analysed with the use of Graph pad^{R} Instat 3 package and employing unpaired t. test to compare the means of flood and non-flood locations, cultivated and market sold samples.

Macroscopy and Microscopy of the Leaves: Macroscopy of the leaf was carried out with the aid of the naked eyes. Preparation of specimens for microscopical examinations was carried out by standard methods for the sections and powdered leaves [37, 43]. All observations were made using a binocular microscope.

RESULTS

Out of the forty-one leaf samples collected from cultivated sources, 71% were infested with parasites while the remaining were free showing that approximately three-quarter of the sample population contained intestinal parasites (Table 1, Fig. 3). Samples collected from the flood affected locations were compared with samples from the flood free locations; the two most contaminated leaf samples were from flood affected areas. Both flood and non-flooded areas were equally (about 70%) contaminated (Table 1, Fig. 4). When samples from cultivated sources were compared with those from the market the level of the parasitic contamination was 19% higher in market samples (Table 1). Generally, *A. lumbricoides* was the highest in terms of abundance and prevalence rate in the study area as well as and in the intensity of infestation in the contaminated samples while *S. stercoralis* was the least from cultivated samples (Tables 1, 2 & 3). *A. lumbricoides* and *T. trichuria* were higher in prevalence and abundance as well as in intensity in the flooded locations compared to the flood free locations (Table 3). Hookworms were not location dependent, while *E. histolytica* and *S. stercoralis* were present only in flood free locations (Table 3). Hookworms were absent in market samples, while other parasites were more abundant and more prevalent in samples obtained from the market than those from cultivated sources. The intensity of infestation in the contaminated samples

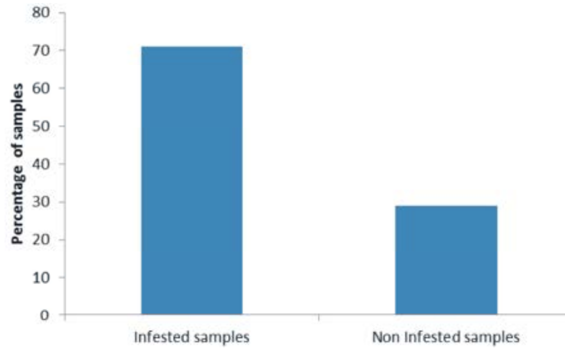


Fig. 3: Population of infested and non-infested *Telfairia occidentalis* leaf samples from cultivated sources

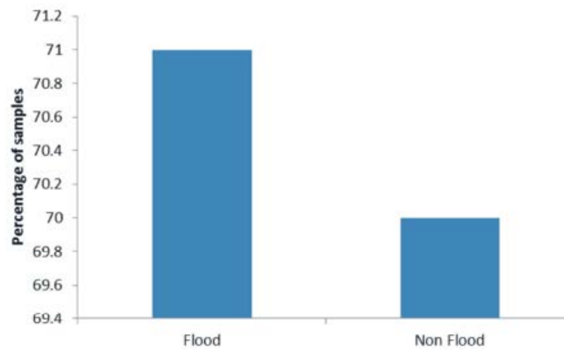


Fig. 4: Percentage of Infested *Telfairia occidentalis* leaf samples from flood and non-flooded areas

Table 1: Intestinal parasites in *Telfairia occidentalis* Leaf samples

SAMPLE	Sample size	Number of Samples infested	Ova of *Ascaris lumbricoides*	Hookworm	Ova of *Trichuris trichuria*	Cysts of *Entamoeba histolytica*	Larvae of *Stongyloides stercoralis*	Total number of parasites
Flood / Cultivated	14	10 (71%)	15 (75%)	1 (5%)	4 (20)	0 (0%)	0 (0%)	20
Mean			1.07	0.07	0.29	0	0	
Non flood / Cultivated	27	19 (70%)	12 (48%)	2 (8%)	4 (16%)	6 (24%)	1 (4%)	25
Mean			0.44	0.07	0.15	0.22	0.04	
Total Cultivated Flood/Non Flood	41	29 (71%)	27 (60%)	3 (7%)	8 (18%)	6 (13%)	1 (2%)	45
Market	10	9 (90%)	7 (47%)	0 (0%)	3 (20%)	4 (27%)	1 (7%)	15
Mean			0.7	0	0.3	0.4	0.1	

Table 2: The Prevalence, Intensity and Abundance of the Intestinal Parasites in *Telfairia occidentalis* Leaves

Parameter	Ova of *Ascaris lumbricoides (%)*	Hookworm *(%)*	Ova of *Trichuris trichuria (%)*	Cysts of *Entamoeba histolytica (%)*	Larvae of *Strongyloides stercoralis (%)*
Prevalence	44	7	17	12	2
Intensity	150	100	114	120	100
Abundance	66	7	20	15	2

Table 3: Percentage Comparative Prevalence, Intensity and Abundance of the Intestinal Parasites in *Telfairia occidentalis* Leaves in Flooded and Flood free Locations in Amassoma

	Ova of *Ascaris lumbricoides*		Hookworm		Ova of *Trichuris trichuria*		Cysts of *Entamoeba histolytica*		Larvae of *Strongyloides stercoralis*	
Location	Flood	Flood Free	Flood	Flood Free	Flood	Flood Free	Flood	Flood Free	Flood	Flood Free
Prevalence	57	37	7	7	21	14	0	19	0	4
Intensity	225	120	100	100	133	100	0	120	0	100
Abundance	107	44	7	7	29	15	0	22	0	4

Table 4: Percentage Comparative Prevalence, Intensity and Abundance of the Intestinal Parasites in *Telfairia occidentalis* leaves from the market and cultivated sources

	Ova of *Ascaris lumbricoides*		Hookworm		Ova of *Trichuris trichuria*		Cysts of *Entamoeba histolytica*		Larvae of *Strongyloides stercoralis*	
Source	Cultivated	Market	Cultivated	Market	Cultivated	Market	Cultivated	Market	Cultivated	Market
Prevalence	44	50	7	0	17	30	12	30	2	10
Intensity	150	140	100	0	114	100	120	133	100	100
Abundance	66	70	7	0	20	30	15	40	2	10

Table 5: Unpaired t. test between Intestinal Parasites in Flood and Non-Flood Locations

	Ova of *Ascaris lumbricoides*		Hookworm		Ova of *Trichuris trichuria*		Cysts of *Entamoeba histolytica*		Larvae of *Strongyloides stercoralis*	
Location	Flood	Non Flood	Flood	Non Flood	Flood	Non Flood	Flood	Non Flood	Flood	Non Flood
Mean	1.0714*	0.4444	0.0714	0.0741	0.2857	0.1481	0	0.2222	0	0.0370
Standard Deviation (D)	1.269	0.6405	0.2673	0.2669	0.6112	0.3620	0.000	0.5064	0.000	0.1925
Population Size (N)	14	27	14	27	14	27	14	27	14	27

Key: *P < 0.05 (between the same parasite from the two different locations)

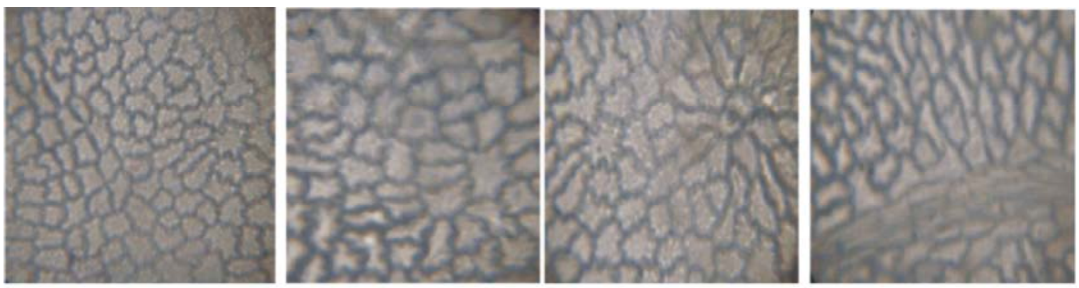
Table 6: Table 5. Unpaired t. test between Intestinal Parasites from Cultivated and Market Sources

	Ova of *Ascaris lumbricoides*		Hookworm		Ova of *Trichuris trichuria*		Cysts of *Entamoeba histolytica*		Larvae of *Strongyloides stercoralis*	
Location	Cultivated	Market	Cultivated	Market	Cultivated	Market	Cultivated	Market	Cultivated	Market
Mean	0.6585	0.7000	0.0732	0.0000	0.1951	0.3000	0.1463	0.4000	0.0244	0.1000
Standard Deviation (D)	0.9383	0.8233	0.2637	0.0000	0.4593	0.4830	0.4220	0.6992	0.1562	0.3162
Population Size (N)	41	10	41	10	41	10	41	10	41	10

Key: P > 0.05 (between the same parasite from the two different locations)

Table 7: Macroscopy of *Telfairia occidentalis* leaf

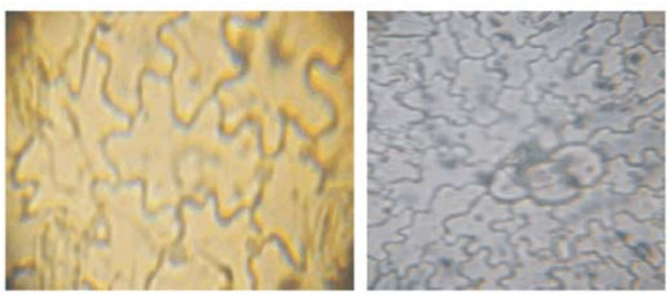
1	CHARACTER	DESCRIPTION
2	Composition/leaf type	Simple, palmate
4	Shape	Elliptical, round/ovate
5	Size range	Length = 11.2 cm, Breadth = 8.5 cm
7	Margin	Serrate
8	Apex	Acuminate
9	Base	Cordate, symmetrical
10	Habit	Shrub
11	Surface	Glabrous on both surfaces
12	Colour	Dark green (upper surface), light green (lower surface)
13	Texture	Papery on both surfaces



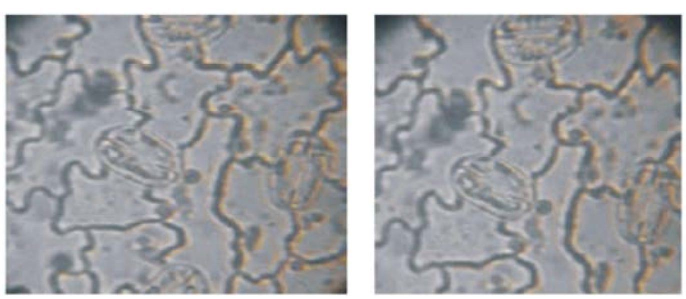
Wavy, polygonal epidermal cells devoid of stomata

A B C D

Plate 2: Microscopy of the upper surface of *Telfairia occidentalis* Leaf (A-D)



E. Wavy epidermal cells F. An isolated glandular trichome



G H

Wavy epidermal cells with Anomocytic stomata

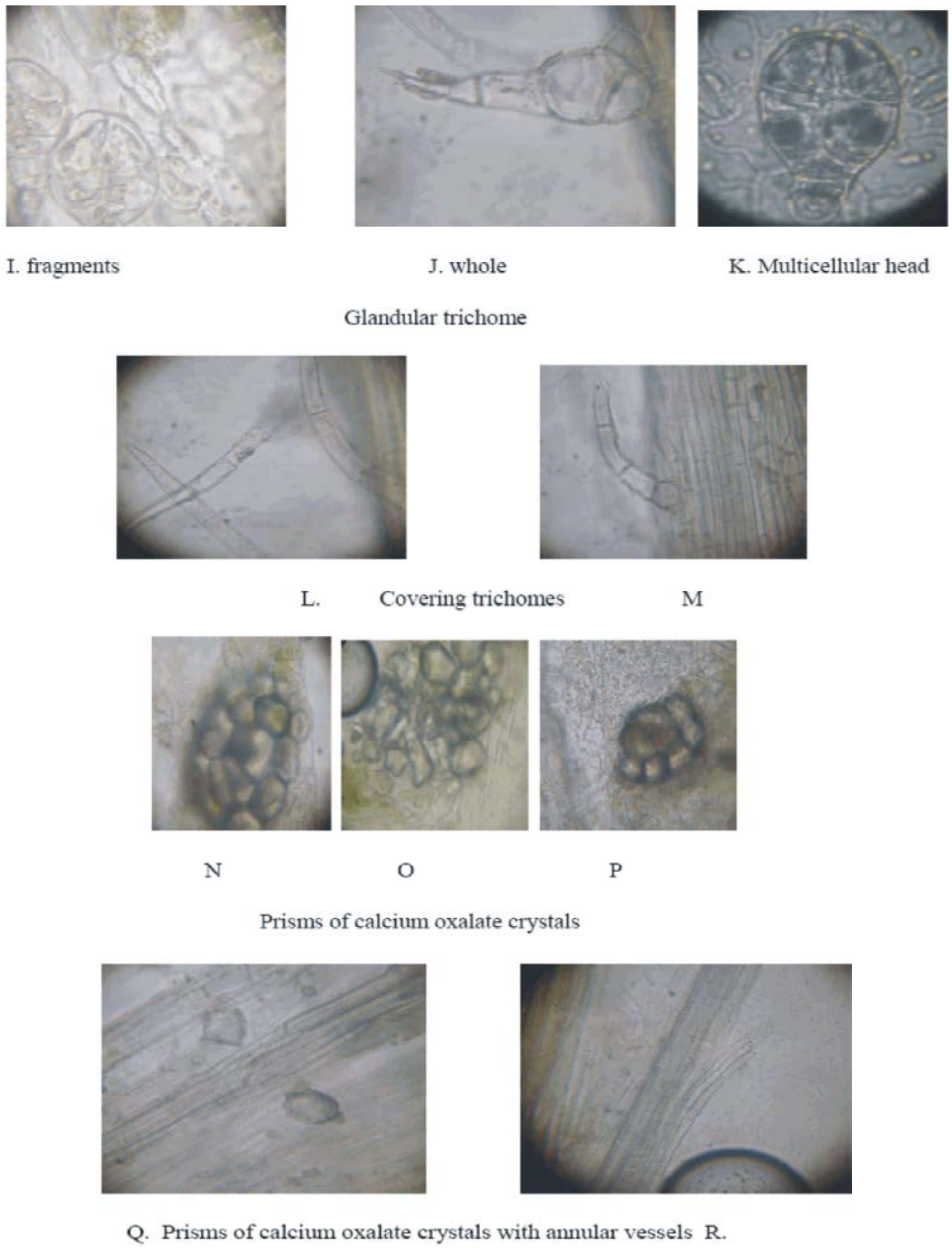


Plate 3: Microscopy of the Lower surface of *Telfairia occidentalis* Leaf (E-R)

was approximately equal (Table 4). The macroscopy and microscopy of the leaves are as presented in Table 7 and respectively.

The unpaired t. test showed significant difference between *A. lumbricoides* ($p < 0.05$) in flood and flood free areas; however, there was no significant difference among the other parasites in these two locations (Table 5).

There was no significant difference in the parasitic loads from cultivated and those sold in the market (Table 6).

Macroscopically, the leaf is green in colour, the upper side being somewhat darker than the lower. It is simple, palmate, elliptical, round to ovate in shape with acuminate apex, the base is cordate and symmetrical, both surfaces are glabrous with papery texture. The length of the leaf ranges between 8.5 -11.2 - 14 cm while the breadth is 6.5 - 8.5 - 12 cm (Table 7).

The upper epidermis was wavy and was devoid of stomata, some cells had almost straight anticlinal wall (Plate 2; A-D). The lower epidermis showed wavy

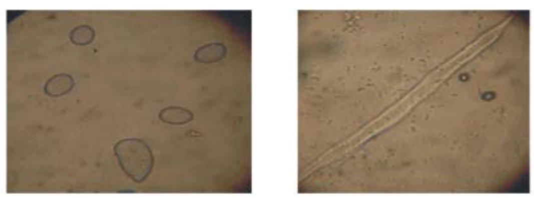
S T

Transverse section showing V: vascular bundles, C: collenchyma, L:lower and U: upper epidermis, T: covering trichome

Plate 4: Microscopy of the Transverse section of *Telfairia occidentalis* leaf through the midrib (S-T)

U. covering trichomes V X Y

W - Y. prism of calcium oxalate crystals



Z. Starch granules Z1. Styloid crystal in crystal idioblast cell

Plate 5: Microscopy of the powdered leaf of *Telfairia occidentalis*

epidermal cells with anomocytic stomata, starch grains were present in abundance; 3-4 celled covering trichomes were numerous and a few glandular trichomes were observed. Numerous prisms of calcium oxalate crystals (Plate 3; E-R), vascular bundles and fibres were also found on the epidermis in the transverse section (Plate 4; S-U). Covering trichomes, prisms of calcium oxalate crystals and fibres were also present in the powdered leaves (Plate 5; V-Y).

DISCUSSION

The data provide the evidence that *T. occidentalis* leaves harbour a wide range of parasitic contaminants. Vegetables have been reported to harbour parasites [43-46] in their leaves. Intestinal parasites on vegetables have been linked with illnesses [46-47], the use of the raw form of *T. occidentalis* as it is commonly employed in ethnomedicine may therefore be associated with outbreaks of diseases as has been reported for other vegetables in many countries of the world [48-50]. Millions of people especially in the developing countries have suffered from parasitic infections from vegetable consumption [51]. Contaminations of the samples collected from cultivated sources may have been introduced from soil, faeces (human and animal origin), sewage or unhygienic processing of the material [52-53]. The intestinal parasites were more abundant in flood affected locations; contamination could therefore be ascribed to sewage contamination during flood. The most prevalent intestinal parasite was *A. lumbricoides* and this is consistent with some findings from vegetables and fruits sold in some markets in some parts of Nigeria [54-55]. Also, the parasitic infestation was more in the samples obtained from the market. This might be due to improper handling by vendors and the likelihood of the use of unsafe water for rinsing as well as for sprinkling to keep them fresh [56]. The highest number of parasites per sample was found to be *A. lumbricoides* and was also observed in the samples from a flood affected location. *E. histolytica* and *S. stercoralis* were completely absent in the flood affected areas (Tables 1 and 4), which may indicate that the flood washed them away or that they could not survive it. The result shows that those sold in the market and the cultivated were from similar sources. Conclusively, flood affected locations had higher number of intestinal parasites than those from flood free areas although the difference was only significant in *A. lumbricoides.* There is therefore the need for thorough cleaning of the leaves with clean water prior to use, as these parasites pose a health threat. It is known that *Ascaris* infection affects the physical and intellectual status of children; a report revealed that it causes low intelligent quotient, significantly low body weight and lower haemoglobin concentration [57-58].

The microscopical characterization of the transverse section, the upper and lower surface which were not reported in previous work [37] were studied in detail. A distinct diagnostic feature is the styloid crystal in crystal idioblast cells. The idioblast has a single large styloid with sharply pointed ends. It has a role in defence of the plant. The diagnostic microscopical characters may be used in the differentiation of adulterants and proper identification of the plant materials.

CONCLUSION

Ascaris lumbricoides has been shown to be abundant in Amassoma and therefore poses a major public health concern. This is an indicator of a negative effect on growth of undernourished children. Its possible minute role in causing intestinal obstruction and low intelligent quotient cannot be neglected. There is therefore the need for awareness on provision of good sanitation and general proper hygiene in handling vegetables for consumption.

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