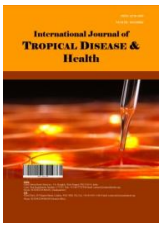

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The Potential Risk of the Used *Thaumatococcus danielli* (Benn) Leaves in Food Packaging Process in Transmission of Antibiotic Resistant Pathogens in Ghana

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Authors' contributions:

This work was carried out in collaboration among all authors. Authors VK, SAA, PAAO, GKN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SAA and PAAO managed the analyses of the study. Authors SAA, PAAO, GKN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Thaumatococcus danielli leaf is a known forest plant species that is widely used by food vendors for food packaging. In this study, the possible risk of *T. danielli* leaf used in food packaging, with its antibiotic-resistant pathogen transmission potential was evaluated. Five hundred leaves were swabbed from vendors at different food joints and were subjected to the routine bacteriological examination. Pure cultures from swabs were biochemically analysed, after microscopic examination. Most bacteria isolates were members of the *Enterobacteriaceae*. Other species included *Staphylococcus aureus,* CoNS (Coagulase-Negative Staphylococci) and *Pseudomonas sp*. The bacterial isolates were tested for their antimicrobial susceptibility towards 11 antimicrobials. As well as some factors such as community hygiene levels and people behaviour and their

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influences on the transmission of these microorganisms were statistically analysed using frequency tables. Most of the food vendors (43%) were between the ages of 31-40. Those with tertiary education were mostly aware (58%) of the possible transmission of pathogens by the leaves. Moreover, the leaves are preferred by food buyers irrespective of their income. Bacteria belonging to sixteen (16) different genera were identified, all of which were resistant to at least three antibiotics. The study showed that *T. danielli* leaves used by food vendors in two popular markets in Cape Coast Metropolis were contaminated and may serve as a possible vehicle for antibiotic-resistant bacteria transmission.

Keywords: Food packaging; Thaumatococcus danielli leaves; Antibiotic resistance.

1. BACKGROUND

Thaumatococcus danielli is commonly known as the "sweet prayer plant" and is a non-timber forest product that is heavily relied on and contributes to the welfare of the rural folks including food production, creation of employment, provision of rural infrastructure and medicinal uses [1]. It is a stemless herbaceous perennial with a rhizomatous root belonging to the family *Marantaceae* (Benth) [1]. The plant produces leaves ranging from 25-30cm wide by 40-45cm long on a leaf stalk of about 2-3m long and it is cultivated in West Africa mainly for its leaves despite the numerous uses associated with its fruits, stalk and rhizome [1].

T. danielli leaves are used for roof thatching and the sap obtained from the leaves is used as an antidote for venoms, stings and bites [2]. The seed of *T. danielli* produces jell and provides a substitute for agar used in microbiological studies [3]. The stalk is used for weaving baskets, mats, fish traps, ornamental bags and as a sponge [4]. Furthermore, it is used to wrap food such as "Moi-moi" (beans pudding), "Adibi", "Aboloo" (household delicacies in Ghana made from fermented maize) before boiling and for packaging/serving already cooked foods like rice, "kyinkafa de waakye'' (a mixture of rice and beans with a characteristic reddish-brown colour).

The preference for this leaf in food wrapping is of high demand by most Ghanaians because research has revealed that the phytochemical constituents such as alkaloids, flavonoids, cardiac glycosides, tannins, as well as proteins, fats and minerals present in this leaf, impact a particular taste, colour and aroma to food [5]. Although plastics are widely preferred and used for their characteristics and versatility in applications, their usage has reached alarming proportions in that it poses threats to the natural environment and some extent, terrestrial habitats [6]. As such, there is a roaring comeback as news has spread of the health dangers associated with packaging/serving hot food in polythene [7]. Although *T. danielli* leaves seem to improve the taste of food, they could potentially serve as a mode of transmitting disease-causing microbes. While antimicrobial resistance has steadily been increasing, for example, with Extended-Spectrum Beta Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella* spp [8], contaminated leaves for packaging food can contribute to the transmission of these multi-drug resistant microorganisms in the community [9]. Immuno-compromised individuals further stand the risk of acquiring opportunistic infection, through handling and eating from contaminated leaves used in food packaging [10].

Though these leaves are widely used, information about their safety and documentation is spotty. Given this, our study sought to evaluate the knowledge of the food vendors on the proper use of the leaves of *T. danielli* together with the potential risk of antibiotic-resistant pathogen transmission of the leaves.

2. METHODOLOGY

2.1 Study Area

The study was conducted in two selected market communities in the Cape Coast Metropolis of the Central Region of Ghana: Kotokuraba market and Abura market. The Kotokuraba market is the premier economic hub of the region located at 5^{o}06'56.5''N, 1^{o}16'56.4''W, with all major trading stores located in and around it. The study was conducted between February 2018 and May 2018. Abura is the second-largest market trading centre of the Metropolis located at 5.1139^{o} N, 1.2597^{o} W. Food vendors who included the use of the leaves of *T. danielli* in packaging food in these two markets were included in this study.

2.2 Study Design and Data Collection

Reconnaissance surveys were initially conducted in selected areas to identify the use of *T. danielli* leaves by food vendors. A cross-sectional study with a randomized sampling technique was adopted for the study. The demographic characteristics of consenting food vendors and customers from the Kotokuraba market and Abura market were taken using a well-structured questionnaire. Administered questionnaires were orally interpreted and translated into the local languages “Twi” and “Fante” to respondents who could not speak English.

2.3 Sample Collection

T. danielli leaves from 100 food vendors that have undergone the various cleaning methods; be it wet, dry or salty water cleaning were swabbed using sterile cotton swab sticks. In the two study areas, 5 swabbed samples were taken from each food handling respondent. Swabbing was carefully done to avoid interference with true practical results. Swabs were transferred immediately on ice to the Department of Biomedical Science laboratory within an hour of collection for bacteriological analysis. Written consent was obtained from the food vendors before swabs were taken.

2.4 Laboratory Investigation

2.4.1 Bacteriological examination

This aspect of the study was conducted in the Microbiology Laboratory of the Department of Biomedical Sciences, the University of Cape Coast. Swabs were directly inoculated onto prepared peptone water, followed by plating on MacConkey agar and Blood agar after which they were incubated for 12 to 24 hours at 37ºC. Following this, the morphological appearance of colonies was noted and recorded. Distinct colonies of all microorganisms were subcultured on MacConkey and Blood Agar and incubated as done previously.

2.4.2 Identification of bacterial isolates

The identification of bacteria growth was done by routine standard laboratory methods as described by Cheesebrough, 2006 [11]. The isolates were identified based on the use of special media such as mannitol salt agar, salmonella shigella agar, their colony morphology, hemolysis on blood agar, Gram stain and biochemical tests including catalase test, oxidase test, triple sugar iron test, citrate test, indole test, motility test and urease test.

2.5 Antibiotic Susceptibility Testing

The antibiotic sensitivity of the pure isolates was tested against tetracycline (10µg), cotrimoxazole (25µg), gentamicin (10µg), tetracycline (10µg), chloramphenicol (10µg), ceftriaxone (30µg), cefuroxime (30µg), cefotaxime (30µg), Ampicillin (10µg), penicillin (1.5µg) and erythromycin (25µg) using Kirby-Bauer antibiotics disc method. A colony of each test organism was picked and immersed in peptone water. The turbidity of the suspension was compared against a reference 0.5M McFarland standard and further plated on Mueller-Hinton agar. The discs impregnated with standardized amounts of antibiotics were gently pressed onto the surface of the agar with the aid of a sterile loop. The plates were incubated overnight at 37ºC. The resistance and susceptibility of the various bacteria isolates were recorded based on the Clinical and Laboratory Standard Institute [12].

2.6 Statistical Analysis

The data were entered into the Statistical Package for the Social Sciences version 21 (SPSS Inc, IBM), and expressed in tables. Graph pad prism 7 was used to express the data as bar charts.

3. RESULTS

3.1 Demographic Characteristics of the Sample Population

In this study, the income levels of those buying food from these vendors were taken, considering their preferred packaging (Table 1). Most of the food vendors were aged between 31 and 40 (43%) whereas the least (10%) were aged between 51 and 60. Most (49%) had completed senior high school and most (36%) used napkins in cleaning the leaves for packaging food (Table 1).

3.2 Characteristics of the Food Vendors and their Knowledge of Disease Transmission

Most of the food vendors that had junior high school education (JHS) (40%), senior high

school education (SHS) (45%) and tertiary education (42%) were found to be between the ages of 31 and 40 (Table 2). The least were mostly those between the ages of 51 and 60 where none had tertiary education and only 6% had senior high school education (Table 2). Moreover, most of the food vendors with tertiary education (58%) knew the pathogen transmission potential of the leaves but only 16% of those with JHS education and 25% of SHS graduates knew (Table 2).

Table 1. Demographic characteristics of street food vendors and food consumers

Street food vendors	
Age group	**Frequency**
20 – 30	17
31 – 40	43
41 – 50	30
51 – 60	10
Level of Education	
JHS	25
SHS	49
Tertiary	26
Cleaning Method	
Napkin	36
Ordinary Water	32
Salty water	32
Location	
Abura Market	50
Kotokuraba Market	50
Food Consumers	
Level of Income	
High income	18
Middle income	93
Low income	189
Location	
Abura Market	150
Kotokuraba Market	150

3.3 Level of Income of Buyers and their Food Packaging Preference

From Fig. 1, almost all the customers patronizing food from the food vendors preferred to have their food packaged in *T. danielli* leaves; 85%, 79% and 72% for those with low income, middle income and high income respectively. Rubber polyethene was the least preferred among high income, middle income and low-income food buyers. More high-income earners (16%) preferred styrofoam for their food packaging compared to the middle (11%) and low-income earners (10%).

3.4 Cleaning Methods with the Age and Education Levels of Food Vendors

T. danielli leaves cleaned with salty water (18%) had the least contamination followed by those cleaned with ordinary water (25%). Leaves cleaned with only napkins were highly contaminated (57%) (Table 3). Most of the food vendors who had tertiary education (65%) employed the use of salty water in cleaning the leaves with none of them using napkins. Most of the food vendors with SHS education (43%) used napkins whereas only two (2) with JHS education used salty water (8%). Older vendors (50-60 years) employed the salty water cleaning method. A few young food vendors (21-30 years) (12%) used salty water compared to 19% of those between 31 to 40 years also used salty water. About 17% of those between 41 and 50 years used ordinary water (Table 3).

3.5 Percentage Occurrence of Bacteria Isolates

Most of the isolated bacteria were gram-negative with a few gram-positive bacteria (Fig. 2). The bacterial percentage recovery were high on the side of *E. coli* (12%), followed by *Staphylococcus aureus* (10.8%), *Proteus sp* (8.5%), *Salmonella sp* (8.1%), *Yersinia spp* (7.8%), *Shigella sp* (7.4%), Coagulase-negative *Staphylococcus* (7.3%), *Moraxella sp* (6.2%), *Klebsiella spp* (5.5%), *Listeria sp* (5.3%), *Streptococcus sp* (5.1%), *Pseudomonas sp* (4.6%), *Hemophilus sp* (4.4%), *Enterococcus sp* (3.7%), *Bacillus sp* (2.8%) with *Lactobacillus sp* (0.7%) being the least encountered (Fig. 2).

3.6 Antibiotic Resistance Patterns of Gram-positive and Gram-negative Bacteria

One isolate representing each genus was tested against selected antibacterial agents. All the gram-negative bacteria were resistant to ampicillin, tetracycline and cefuroxime whereas all the gram-negative bacteria were susceptible to gentamicin except *Pseudomonas* (Table 4). *Pseudomonas sp* was resistant to all the antibiotics except chloramphenicol and cefotaxime. *Moraxella sp* was also resistant to all the antibiotics except for gentamicin. *Yersinia sp*, *E. coli* and *Shigella sp* were resistant to 50% of the antibacterial agents used (Table 4). For the gram-positive bacteria, tetracycline, ampicillin,

penicillin and flucloxacillin were ineffective against all the bacteria. However, gentamicin was effective against most of the bacteria isolates. Co-trimoxazole was also effective against most of the bacteria in this study except *Bacillus sp* (Table 4).

Table 2. The education level and their age groups and knowledge of disease transmission

Education level	Age Group				Knowledge of Disease Transmission N (%)	Total
	20 – 30 N (%)	31 – 40 N (%)	41 – 50 N (%)	51 – 60 N (%)		
JHS	8 (32)	10 (40)	0 (0)	7 (28)	4 (16)	25
SHS	5 (10)	22 (45)	19 (39)	3 (6)	12 (25)	49
Tertiary	4 (16)	11 (42)	11 (42)	0 (0)	15 (58)	26
Total	17	43	30	10		**100**

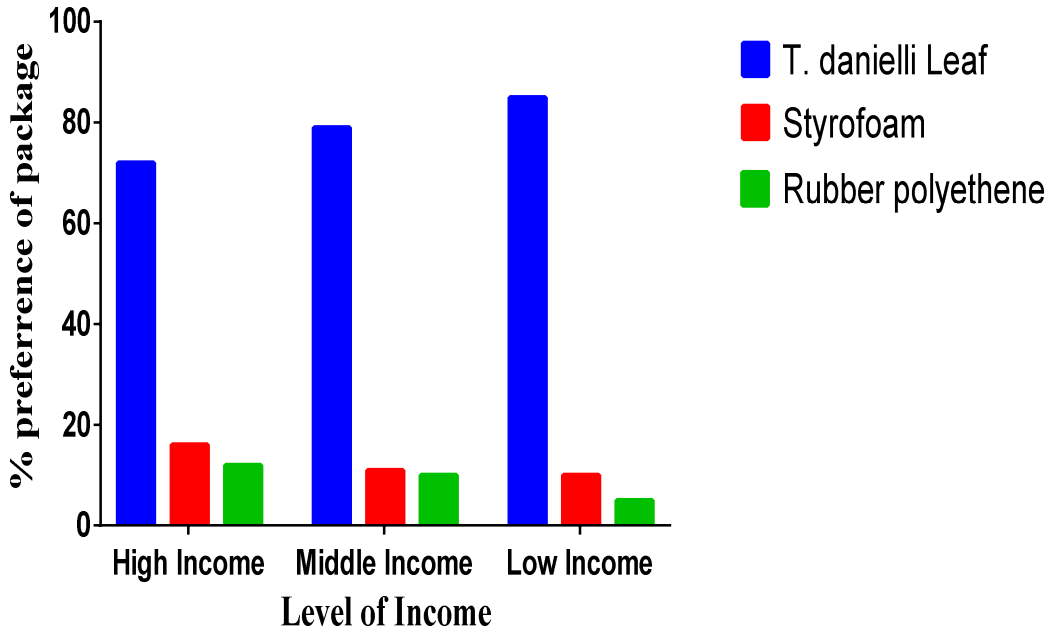

Fig. 1. Level of income of food consumers and their preferred package

Table 3. The Cleaning Methods employed by Street Food Vendors based on their Educational Level and Age Groups

Educational Level	Cleaning Method		
	Napkin N (%)	Ordinary Water N (%)	Salty Water N (%)
JHS	15 (60)	8 (32)	2 (8)
SHS	21 (43)	15 (31)	13 (27)
Tertiary	0 (0)	9 (35)	17 (65)
Age Group			
20 – 30	5 (29)	10 (59)	2 (12)
31 – 40	18 (42)	17 (40)	8 (19)
41 – 50	13 (43)	5 (17)	12 (40)
51 – 60	0	0	10 (100)
Degree of contamination (%)	57	25	18

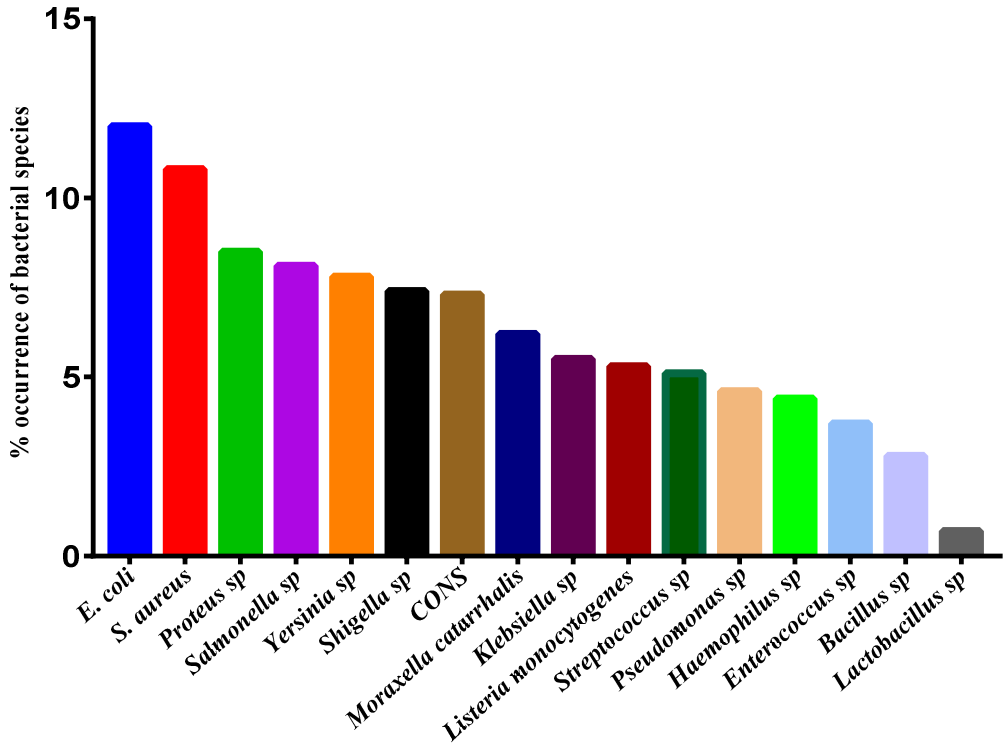

Fig. 2. Diverse percentage occurrence of bacterial isolates swabbed from the leaves surface.

Table 4. Antimicrobial susceptibility tests of bacteria isolates

	Antibiotics for gram-negatives							
Isolates	**AMP**	**TET**	**COT**	**GEN**	**CRX**	**CHL**	**CTR**	**CTX**
Yersinia sp.	R	R	R	S	R	S	S	S
Haemophilus sp.	R	R	S	S	R	S	S	S
Pseudomonas sp.	R	R	R	R	R	S	R	S
Moraxella sp.	R	R	R	S	R	R	R	R
Klebsiella sp.	R	R	R	S	R	R	S	R
E. coli	R	R	R	S	R	S	S	S
Shigella sp.	R	R	R	S	R	S	S	S
Proteus sp.	R	R	S	S	R	R	R	R
Isolates	**Antibiotics for gram-positives**							
	TET	**AMP**	**CRX**	**GEN**	**ERY**	**PEN**	**FLX**	**COT**
S. aureus	R	R	S	S	R	R	R	S
Bacillus sp.	R	R	S	S	S	R	R	R
Listeria sp.	R	R	R	S	R	R	R	S
Streptococcus sp.	R	R	R	S	R	R	R	S
Enterococcus sp.	R	R	R	S	R	R	R	S
CONS	R	R	S	S	R	R	R	S
Salmonella sp.	R	R	R	S	R	R	R	S
Lactobacillus sp.	R	R	S	S	S	R	R	S

Note: S=Susceptibility, R=Resistance, AMP=Ampicillin, TET=Tetracycline, COT=Cotrimoxazole, GEN=Gentamicin, CRX=Cefuroxime, CHL=Chloramphenicol, CTR=Ceftriaxone, CTX=Cefotaxime, PEN=Penicillin, ERY=Erythromycin, FLX=Flucloxacillin, CONS= Coagulase-negative bacteria

4. DISCUSSION

Thaumatococcus danielli leaf is a widely use forest plant species whose leaf is used in food packaging or rapping by food handlers locally, without much knowledge in its potential to transmit microbes of health importance. Education is a strong factor in improving population health by building on the individual capacity to process and understand risks related to several infections [13]. Adversely, a low educational level leads to a lack of knowledge, which hinders individuals from analyzing their behavioural choices, to put one at health risks. In this study, 58% of the responding food vendors [14] with tertiary education knew about the possible transmission of microbes from leaves to an individual (Table 2). This is an indication that such groups were more enlightened about the risk factors associated with the spread of infections and as a result, 65% of them employed the "salty water" method of cleaning with a lower degree of contamination (18%). Salt or salt solution over the years, has been a traditional method for disinfection and food preservation. Whiles some studies have revealed the antimicrobial properties of salt [15], others have doubted the ability of salt to inhibit microbial growth [14,16] as obligate and facultative halophiles would survive in the presence of salt. Comparatively, Junior High School (J.H.S) graduates had inadequate knowledge of microbial transmission and prevention (16%), using mainly napkin for cleaning leaves before use and thus led to a 57% bacteria contamination (Table 3).

The cleaning of the leaves is often done at different times and more frequent cleaning has been reported to be associated with a lower prevalence of bacterial contamination [17]. On the contrary, the leaves clean with napkin recorded a wider spectrum of bacteria with the highest bacteria load of 57%, whereas, those cleaned with ordinary water showed 25% bacteria load. As expected, leaves that cleaned with salty water recorded the least bacteria load of 18% (Table 3). The least microbial content of leaves cleaned with salty water could be a result of the hypertonic nature of the water which tends to have the plasmolytic effect on microbes that cannot tolerate salt, corroborated by other studies [18]. Even though the oldest of the food vendors (51 – 60 years) had no tertiary education, they all employed the salty water method of cleaning the leaves (Table 2 and Table 3). This group of vendors was aware of the possible antimicrobial properties of salt as it has been a traditional method used over years had adequate experience with its use.

The demand for food consumption in *T. danielli* leaves was very high among individuals regardless of their income levels (Fig. 1). This could be due to cultural, social or personal reasons as a greater proportion of the vendors believed that the leaves were used in the days of old by their ancestors and that possess no threat to health, unlike the polyethene locally called "take-away" and Styrofoam packs. The few people who preferred the use of Styrofoam packs to leaves were of the view that the leaves were an old-fashioned means of packaging and could be dangerous to one's health when dirty. Plastic and rubber packages have been found to possibly release toxic compounds into foods and have been proven to be carcinogenic [19].

The microorganisms that colonize leaves are dominated by bacteria, usually reaching $1x10^7$ individuals per cm^2, making $1x10^{26}$ bacteria in total on the world's leaf masses [20]. Therefore, it is not surprising that the microbiological techniques employed in this study showed various species of bacteria (Fig. 2) on *T. danielli* leaves. The genera of isolated bacteria were mostly members of the *Enterobacteriaceae* namely; *E. coli*, *Proteus sp, Klebsiella sp, Shigella sp, Salmonella sp* and *Yersinia sp* with others being *Moraxella sp*, *Haemophilus sp and Pseudomonas sp* as shown in Fig. 2. Some Gram-positive bacteria were isolated with *S. aureus* (10.8%) being the most dominant amongst them.

The presence of *E. coli, Klebsiella sp., Salmonella spp.* and *Proteus sp* is an indication of faecal contamination [21]. *Klebsiella spp, Salmonella spp, Proteus sp* and *E. coli* are normal commensal flora and are known to cause opportunistic infections especially in immune-compromised patients as well as diarrhoea [22]. Thus, the presence of these bacteria raises an obvious public health concern. Most of the microbes isolated in this study have been implicated in food spoilage and food-borne diseases [17]. Colonization of the leaves by microbes could occur due to unhygienic and poor food handling practices. This accounts for the presence of respiratory pathogens such as *Moraxella sp*, *Haemophilus influenzae* and *S. aureus*. The environment could be a source of colonization as *Bacillus sp* is commonly found in the soil and also capable of forming spores that

contribute to the resilience of the bacteria [23]. Another factor could be linked to the source of these leaves as they are harvested without adhering to any protocol or technique to ensure the cleanliness of the leaves. Again, poor preservation of the leaves may have contributed to the bacterial load.

Most of the bacteria encountered in this study are members of the normal flora of the skin thus; coagulase-negative *Staphylococcus* and *Staphylococcus aureus*. The abundance of *S. aureus* could be associated with the fact that it is a salt-tolerant bacterium and hence be found on leaves cleaned with salty water [16]. Therefore, more cautious management is needed to prevent foodborne outbreaks from leaves used as packaging materials.

With the antibiotic susceptibility patterns of the bacteria isolated from the leaves, none of the microbes isolated showed resistance to all the antibiotics tested but most were susceptible to at least three of the antibiotics. Generally, all the bacteria isolated in this study were resistant to at least three antibiotics indicating the multidrug resistance of all the bacteria. Broad-spectrum drugs such as ampicillin and tetracycline were ineffective against all bacteria isolated in this study and penicillin and flucloxacillin were also ineffective against all gram-positive bacteria whereas cefuroxime was ineffective against all the gram-negative bacteria. This is so because these drugs are easily accessible and are quickly abused by individuals who find visiting hospitals to be a daunting task. Gentamicin was the most effective drug as it was effective against all the gram-positive bacteria and all the gram-negative bacteria except *Pseudomonas sp.* This is probably because gentamicin is most available as injectables and individuals with expertise can administer these drugs. The limitation to the study was that only one of the bacteria was tested against the antibiotics.

5. CONCLUSION

Microbial infections through food contamination cause one of the largest global health burdens. Therefore, it is important to conduct studies into traditional food packaging materials such as *T. danielli* leaves and how they can be improved to suit modern trends. The results of the current study suggest that *T. danielli* leaves were contaminated with bacteria and may serve as a source of microbial infection if not properly cleaned. Therefore, awareness of vendors on proper hygiene to reduce contamination is needed. The biodegradable leaf plates have vast potential in the international market, which should meet the necessary regulations. To sustain the practice of using leaf packaging and discourage plastic plates, students and the general populace should be educated to realize the importance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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