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RESEARCH ARTICLE

ISOLATION AND IDENTIFICATION OF BACTERIOLOGICAL CONSTITUENTS OF SOME LEAFY VEGETABLES SOLD IN RURAL MARKETS IN ANAMBRA STATE, NIGERIA

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Abstract

Vegetables are a ubiquitous part of everyday meals in Nigeria and have been widely recognized as an essential source of nutrients and dietary fiber for proper health development and wellbeing. The demand for vegetables has increased considerably in recent years. However, research has linked vegetable intake with various pathogenic bacteria that are of public health concern. The purpose of the current study was to isolate and identify the bacterial composition of bitter leaf, fluted pumpkin, and scent leaf commonly sold at various markets in Anambra State. These bacteria were examined with the standard microbiological methods. The serial dilution technique was applied. The result revealed that the samples collected from different sellers containedvarious microbial loads. Bitter leaf contained a higher load of bacteria, while the pumpkin leaf contained a moderate load of bacteria. The lowest plate count was recorded for the scent leaf. The bacteria isolated from the samples were *Bacillus, Pseudomonas species, Erwinia spp,* and *Streptococcus*. Thus, it is concluded that the vegetables sold in the rural markets contain a massive number of bacteria above the world health organization recommendation. Indeed, there should be proper washing of vegetables with clean water and disinfecting the bacteria with sodium chloride to safeguard the health of the final consumer.

Introduction:-

Over the years, vegetables have assumed an essential part of the dietary plan in every society. It reflects a vital food plan and the cornerstone of healthy dietary recommendations (Wallace et al., 2020). Vegetables and fruit are composed of essential nutrients (minerals and vitamins) and fiber primarily required for health and wellbeing. Vegetables are mostly regarded as the fresh edible part of herbaceous plant roots, stems, leaves, or fruits (Damen et al., 2007). Literature abounds that describes vegetables as an essential basis for nutritionand a balanced diet (Boeing et al., 2012; Dennis et al., 2016; Knecht et al., 2015; Okyay et al., 2004;Ogunleye et al., 2010; Yafetto et al., 2019).Scholars have pointed to vegetables as a momentous dietary product needed for human sustenance and daily life(Jana et al., 2020; Liu, 2013; Nti et al., 2011; Slavin & Lloyd, 2012), which has witnessed a substantial increase in production in recent decades (Hess & Sutcliffe, 2018). Research has noted that regular vegetable intake is essential in promoting health and managing body weight (Aughinbaugh, 2015; Dukhi & Taylor, 2018; Myton et al., 2014; Rudra et al., 2019; Ziaei et al., 2020). Dietary guidelines in numerous societies include endorsements for vegetables because of their relevance as a source of a range of vitamins, minerals, and dietary fibers (FAO-WHO, 2017). They are widely associated with reducing the risk of many illnesses (Deribe & Mintesnot, 2016; Hung et al., 2015; Pennington & Fisher, 2009;

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Septembre-Malatere et al., 2018; Ülger et al., 2018). They are also a source of bioactive mechanisms that act through a range of vital means for the appropriate functioning of the body (FAO-WHO, 2017).

The importance of vegetables on healthy living has been widely documented (Asaduzzaman & Asao, 2018; Baidya & Sethy, 2020; Chen et al., 2007; Dhruv et al., 2019; Pal & Molnár, 2021). However, numerous literatureshave associated vegetables with microbial infection (Alemu et al., 2018; Balali et al., 2020; Machado-Morreira et al., 2019; Snyder & Worobo, 2018). Perhaps, the contamination of vegetables is primarily through contact with the soil, introduction of domestic compost as fertilizer, organic fertilizer, and indecorous management during harvest or postharvest processes (Amaechi et al., 2016; Hassibur et al., 2016; Rajwar et al., 2016; Tsado et al., 2015). Nevertheless, the production process to the consumption of a variety of vegetables presents numerous opportunities for bacterial contamination, particularly with the growing consumption of raw vegetables generally driven by the intention of retaining its nutrients. Accordingly, Leff and Fierer (2013) emphasized the probability of most vegetables containing various bacteria, including plant and human-related. For example, research suggests that Salmonella, E. coli, B. cereus, Campylobacter spp., Y. enterocolitis, C. botulinum, and L. monocytogenes are pathogenic bacteria frequently found in vegetables produce (Al-Kharrousi et al., 2016; Alam et al., 2015; Ferroz & Noor, 2019; Heaton & Jones, 2008; Mogren et al., 2018; Warriner, 2005).

Vegetables are widely produced and consumed in Nigeria. Many vegetables, including fluted pumpkin, scent leaf, and bitter leaf, are vastly available in the marketplaces and reflect a potential business for many people (Ibeawuchi et al., 2015). Bitter leaf is one of many leafy green Nigerian vegetables used for culinary and medicinal applications, bitter leaf (Vernonia amygdalina) is bitter tasting, as the name suggests. The fluted pumpkin is an indigenous vine and a member of the cucurbit family. While the fruit isn't edible, the leaves are a famous soup green, and the seeds are high in protein. Fluted pumpkins (Telfairia occidentalis) grow in poor soil and are fairly drought resistant, making them a good choice for any Nigerian. Scent leaf is a native plant with sweet-smelling leaves, making it a welcome addition to the Nigerian gardening-style herb bed. Reputed to cure stomach disorders, scent leaf (Ocimum gratissimum), also known as African blue basil or clove basil, is often added to yam dishes, stews, and pepper soup.

The intricacy associated with the supply of fresh agricultural produce in the marketplacesmight significantly account for the contamination of vegetables. Collecting, processing, and delivering the products describe critical steps in the distribution of vegetables. Indeed, the disparities in these processes may lead to too much unintentional contamination of the vegetables sold in many marketplaces. Hence, the present investigation is aimed to isolate and identify the microbial compositions of some vegetable leave (fluted pumpkin, scent leaf, bitter leaf) sold in the local markets in Anambra State.

Materials and Method:-

Collection of the Samples

Fresh leaves of fluted pumpkin, scent leaf, bitterleaf was purchased from the rural marketplace, placed in separate sterile plastic bags, and conveyed to the laboratory for microbial analysis.

Isolation and identification of bacteria

Pathogenic bacteria were isolated from the vegetables with a serial dilution agar plate using the method described inRuangpan and Tendencia (2004). Biochemical tests encompassing catalase test, indole test, coagulate test, and sugar fermentation test was performed to identify the microbial isolates using the methods described by Cheesbrough (2005).

Result:-

Table 1:- The table shows the standard plate count of the isolated bacteria colonies.

No of plates	Media	Bacteria counts in pumpkins leaf	Bactria counts in the bitter leaf	Bacteria counts in the scent leaf
1	Nutrient agar	$8.0x10^4$	$1.02x10^5$	$6.7x10^4$
3	Nutrient agar	$1.64x10^5$	$9.7x10^4$	$7.2x10^4$

5	Nutrient agar	$1.28x10^5$	$8.9x10^4$	$1.17x10^5$
2	Cled agar	$7.6x10^4$	$6.6x10^4$	$1.10x10^5$
4	Cled agar	$2.7x10^4$	$3.07x10^4$	$7.8x10^5$
6	Cled agar	$5.8x10^4$	$1.14x10^5$	$1.21x10^5$

Table 1 above shows the standard plate count of bacteria colonies isolated from the vegetables (fluted pumpkin, bitter leaf, and scent leaf).

Table 2:- The table shows the morphological characteristic of the isolates, gram reactions, and presumptive organisms.

Morphological characteristics	Gram-positive	Presumptive organism
A	Creamy cloudy surface with uneven figure	Gram-positive cocci	Bacillus spp
	Yellow plane rounded colonies	Gram-negative rod shape	Erwinia spp
	Green colonies on cled agar	Gram-negative rod shape	Pseudomonas spp
	Creamy colonies and smooth beta-hemolytic clusters organized in chains	Gram-positive cocci	Streptococcus spp
B	Pale blue-colored colonies	Gram negative rod	Pseudomonas spp
	Creamy white raised with rough edges	Gram-positive bacillus	Bacillus spp
C	Yellow plane rounded colonies	Gram-negative rod shape	Erwinia spp
	Green colonies on cled agar	Gram-negative rod shape	Pseudomonas spp

KeyA – fluted pumpkin, B – Bitter leaf, C = Scent leaf

Table 3:- shows the biochemical test of the isolates from pumpkin, bitter leaf and scent leaf.

Catalase	coagulation	Indole test	Glucose	Lactose	Fructose	
+	+	-	AG	A	A	Streptococcus
+	+	+	AG	A	AG	Bacillus
+	+	+	A	AG	AG	Erwirria spp
+	-	-	-	A	A	Pseudomonas spp

Key - = Negative, + = Positive, A = Acid, AG = Acid & Gas

Discussion:-

The present study was performed to isolate and identify the bacteriological composition of fluted pumpkin, bitter leaf, and scent leaf sold in the marketplaces. The vegetable samples (pumpkin, bitter leaf, scent leaf) were exposed to bacteria load and isolate identification. The outcome revealed that the produces are influenced by numerous pathogenic bacteria. Sample A (pumpkins) has a moderate bacterium count of $2.6x10^{4,}$ while sample B (bitter leaf) has the highest count of $3.0x10^{4,}$ and sample C (scent leaf) has the lowest count of $2.5x10^4$. Consistent with previous findings (Muhammad et al., 2021; Akinyele et al., 2013; Bae et al., 2011; Mbillia & Shude, 2020; Odu & Okomuda, 2013; Shobha, 2014), the result reported an elevated count of bacteria in the frequently consumed vegetables. The predominant microorganisms identified were of the genus; *Bacillus, Pseudomonas, Erwinia spp, Streptococcus*. However, *Pseudomonas* species occurred in all the vegetables while *Bacillus* were isolated from fluted pumpkin and bitter leaf, *Erwinia* were isolated from fluted pumpkin and scent leaf. *Streptococcus* was only found in fluted

pumpkins. The isolated microorganism from the leaves of the vegetable has been previously implicated as a health concern (Moelering, 2002; Morella et al., 2019; Zeghami et al., 2020), hence, the importance of isolation and identification of the commonly consumed vegetables. Indeed, the study agrees with previous research (e.g., Maia et al., 2013; Ogbonna et al., 2019; Shoba, 2014) that improper product handling from harvesting, storage, processing to distribution accounts for the bacteriological contamination of the vegetables.

Conclusion:-

This study distinguished the profiles of bacteria that produced the pathogenicity of many vegetables sold in the marketplaces. It also demonstrated that bacteria were involved in the spoilage of several vegetables. Injuries, including bruises and cuts occurring during the stage of harvesting or post-harvesting, grading, and packing, might exacerbate spoilage pathogens in the vegetables. Thus, it is recommended that adequate handling of the product is essential in mitigating pathogenic bacterial contamination.

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