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Evaluation of The Use of Terminalia Catappa (Almond Leaves), Theobroma Cacao (Cocoa Leaves) and Thaumatococcus Daniellii (Miracle Berry Leaves) for Wrapping Different Food Products-Impacts on Safety, Sensory Properties and Health Benefits

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Abstract

Background: *The aim of this research was to evaluate the effects of leaf packaging materials (Terminalia catappa; Theobroma cacao and Thaumatococcus daniellii) on the quality of selected food products where they are traditionally used as short-term packaging materials.*

Methods: *Antimicrobial properties of the three leaf extracts on spoilage and pathogenic microorganisms were studied using standard methods. GC-MS analysis was carried out on the leaves and food products wrapped with them after processing.*

Results: *All the leaf extracts showed antimicrobial properties against Escherichia coli, Staphylococcus aureus, and Salmonella typhi except for T. danielli that was not very effective against Bacillus cereus but was quite effective against the other microorganisms. Almond leaves transferred phthalic anhydride and oleic acid up to 90%) to the corn meal concentrate (Agidi) wrapped in it. Gamma sitosterol was found in the "ugba" product wrapped with cocoa leaves. T. daniellii leaves transferred phthalic anhydride (68.5%); Bi-(-ethylhexyl) phthalic acid (87.22%) and gamma sitosterol (91.8%) to the food product (bean pudding). Gamma sitosterol possess antihyperlipidaemic activity while phthalic anhydride possesses anti-viral properties.*

Conclusion: *Terminalia catappa, Theobroma cacao and Thaumatococcus danielli leaves possess anti-microbial properties against food spoilage and pathogenic microorganisms. Bioactive compounds in the leaf packages were transferred to the food products during domestic processing. Some of these compounds promote healthy living.*

Keywords: Leaf-Packages, Terminalia Catappa, Theobroma Cacao, Thaumatococcus Daniellii, Anti-Microbial Properties, Volatile Compounds

1. Introduction

Leaves are used in traditional food packaging all over the world [1]. They protect the food from physical and environmental contamination and damage. They provide containment to facilitate transportation, distribution and storage and add flavor to the food product [2]. The food industry has to develop active, intelligent and innovative food packaging techniques to meet consumer demands for healthy and quality foods [3, 4] and enhance product quality while extending shelf-life. Emerging techniques are improving the passive aspects of food packaging systems, such as thermal stability, barrier effectiveness, and mechanical strength [5]. The use of plants, biodegradable and nano-materials in sustainable food packaging help mitigate its negative environmental impact. By integrating functional and environmentally friendly, packaging technologies, a multipurpose food packaging system can be developed that maintains the integrity of all its components,

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thereby promoting technological advancement [6].

Functional packaging can be used to enhance the status of specialized products to meet the nutrition requirements of vulnerable populations (infants, young children, the elderly and the sick). Medical foods formulated liquid diets, foods for special dietary use, infant formulas, and natural health products can be enhanced with packaging materials that improve their health benefits. The purpose of food packaging is to (a) hold foods, keep them clean and secure (b) protect food during distribution and storage, providing a barrier to contaminants and damage by pests (c) to make handling during production, distribution and storage convenient (d) to provide an identity for the food and ensure that it is properly stored and used [7]. Plant leaves are traditional food packaging materials which are relatively cheap and available. They are used for products that are quickly consumed. Banana and plantain leaves are used to package cheese. Maize leaves are used to wrap corn paste. Some leaves may be woven into bags or baskets and used to carry meat or vegetables. Cellulose, polyethylene (or polythene), propylene and other films are popular as food packaging materials. Biopolymer-based packaging materials are becoming interesting due to their biodegradability, renewability, and biocompatibility. Many biopolymers—such as starch, chitosan, carrageenan, polylactic acid, etc. have been investigated for their potential application in food packaging [8]. Reinforcement agents such as nanofillers and active agents improve the properties of the biopolymers, making them suitable for active and intelligent packaging. Cellulose, starch, polylactic acid, polybutylene adipate and terephthalate, are currently used in the packaging industry [9]. This original article describes the research findings while using three plant leaves as short-term packaging materials for selected food products. It provides the antimicrobial effects of plant leaf aqueous and ethanol extracts on pathogenic and spoilage microorganisms. It reports the major volatiles found in the plant leaves and their level of interaction with the food products wrapped in them. Many plant leaves are eco-friendly, biodegradable, and nontoxic, packaging materials. The unique properties of leaves as packaging materials keep them relevant despite research progress and innovations in food packaging.

2. Materials and Methods

The three leaves (Figure 1) were procured from the wild, cleaned and sanitized with ethanol. They were extracted with water (almond leaves and cocoa leaves after blanching for 5 min), while T. catappa leaf extract was made with ethanol. The inhibitory effects of the extracts on spoilage and pathogenic microorganisms (Bacillus cereus, Staphylococcus aureus, Salmonella typhi and Escherichia coli) were determined. The minimum inhibitory concentrations were recorded. The leaves were used to wrap the different food products (as appropriate). GC-MS was used to determine the volatiles found in the leaves and those found in the food products wrapped with the leaves after domestic processing. This method was used to establish the compounds that were transferred from the packaging materials to the food products.

T danielli ***T. catappa*** ***T cacao***

Figure 1: (Thaumatococcus Danielli; Terminalia Catappa; Thebroma Cacao)

2.1. Testing for The Antimicrobial Properties of Leaf Extracts

In line with the ICFMS method [10], the microbial disc technique was used. In this technique, the leaf extract was prepared in three concentrations which were 100% (100), 1:10 (10-1) and 1:100 (10-2). From the overnight culture, 0.1 ml of each organism was taken and put into 9.9 ml of sterile distilled water to get (1:100) 10-2 M inoculum concentration of the organism. From this dilution, (10-2 M), 0.2 ml was taken into the prepared sterile nutrient agar, cooled to about 40-45 ºC, poured into sterile petri-dishes and allowed to solidify for about 45-60 min. Using a sterile cork-borer of 8 mm diameter, wells were made according to the number of the test tubes for the experiment. For each of the leaf extract, eight wells were made (5mm in diameter and about 2cm apart). The graded concentrations of the extracts were put into the wells accordingly including the controls. Duplicate samples were used. The plates were left on the bench for about 2 h to allow the extract to diffuse properly into the nutrient agar i.e. pre-diffusion.

The plates were afterwards incubated for 18-24 h at 37 ºC. Using Whatman N0 1 Filter paper. Microbial testing discs were prepared and using 0.5ml of each of the extract concentrations the disc was impregnated or infiltrated with the extract concentrations and specifically labeled as such on the disc arm. Benzyl penicillin (Crystalline penicillin G) was used as control. The extract saturated discs were placed inside the petri dish of specific microorganisms growing inside specific selective media in their exponential growth phase. All the plates were incubated in a thermostatically controlled incubator set at 37 0C for 24-48 h respectively except the PDA plates which were incubated at a room temperature of 20-25 0C for 2-3 days because the growth performance of fungi is best at ambient temperature and very poor at temperatures above ambient (above 300C).

2.2. Inhibition of Spoilage and Pathogenic Microorganisms by The Leaf Extracts

At the end of the secondary incubation period, the plates were brought out for observation and measurement. Using sterile forceps, the discs were carefully removed from their plate culture and by means of a pair of dividers and meter rules, the diameter/length in millimeters of the areas or zones where the extracts cleared the microbial growth were determined and recorded. These zones were termed zones of inhibition. The diameter zone of inhibition (mm) was measured using a transparent ruler. The zero end of the ruler was placed from one end of the zone of inhibition to another, ensuring that the ruler divides the well into two equal halves (passing through the center of the wells).

The minimum inhibitory concentration (MIC) of each extract was observed and recorded. The area of least inhibition or microbial clearance was also noted with its corresponding extract concentration. This least extract concentration was termed minimum inhibitory concentration (MIC) of the extract. MIC therefore can be defined as the minimum concentration of any agent or extract that can cause inhibition of growth of microorganisms. These same procedures were repeated using benzyl penicillin as control. The plant leaf extract and the specific concentrations that performed exactly like penicillin were noted and graded according to their different degree of activities against different microorganisms.

2.3. Determination of Volatile Compound

2.3.1. GC-MS Analysis

GC-MS analysis was carried out on GC Clarus 500 Perkin Elmer system (Model; QP 2010 series, Shimadzu, Tokyo, Japan) comprising of a AOC-20i auto-sampler and gas chromatography interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30×0.25 mm ID × 1 μm df, composed of 100% dimethylpoly diloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 μI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature of 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. The total GC running time was 36 min.

3. Interpretation

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) Abuja, having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The concentrations of the identified compounds were determined through area and height normalization.

4. Results and Discussion

4.1. Inhibitory Effects of Leaf Extracts on Spoilage and Pathogenic Microorganisms

The leaves used to wrap the food products are shown in Figure 1. Table 1 shows the inhibitory effects of these leaf extracts on *Bacillus cereus*) food spoilage organism), *Escherichia coli* (pathogenic organism), *Staphylococcus aureus* (pathogenic organism) and *Salmonella typhi*. (a pathogenic organism).

Plant leaf Extract	Microorganisms	Activity	Zone of inhibition (mm)	Minimum inhibitory concentration (MIC)
Cocoa leaf extract	*Bacillus cereus*	+++	120	10^{-2}
10^{0}		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}		+	30	
	Escherichia coli			
10^{0}		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}		+	30	
	Staphylococcus aureus			

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10^{0}		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}		+	30	
	Salmonella typhi			
10^{0}		+++	120	
10^{-1}		++	60	
10^{-2}		+	30	
Almond Leaf Extract	*Bacillus cereus*			
10^{0}		+	30	10^{-0}
10^{-1}		-	0	
10^{-2}		-	0	
	Escherichia coli			
10^{0}		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}		+	30	
	Staphylococcus aureus			
10^{0}		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}		+	30	
	Salmonella typhi			
10^{0}		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}		+	30	
T. danliella leaf extract				
	Bacillus cereus			
10^{0}		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}		+	30	
	Escherichia coli			
		+++	120	10^{-2}
		++	60	
		+	30	
	Staphylococcus aureus			
10^{0}		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}		+	30	
	Salmonella typhi			
10^{0}		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}	+	+	30	

Note: (-) = Negative activity or NO action; (+) = Positive activity or mild activity; (+++) = Heavy activity; (++) = Moderate activity; 100 = Undiluted extract; 10^{-1} = 1:10 (1 in 10) dilution of extract; 10^{-2} = 1:100 (1 in 100) dilution of extract; MIC= Minimum inhibitory concentration

Table 1: Inhibitory Potentials of Plant Leaf Extracts on Selected Spoilage and Pathogenic Microorganisms

4.2. Inhibition of Spoilage and Pathogenic Microorganisms by the Leaf Extracts

Salmonella typhi causes typhoid fever which is spread through contaminated food or water. Once ingested, they multiply and spread into the bloodstream. B. cereus causes two types of gastrointestinal illnesses by producing a toxin with an emetic (vomiting) symptom and another one with a diarrhoeal symptom. It is a common cause of gastroenteritis globally. Escherichia coli is a normal intestinal flora of healthy people and animals. Many types of E. coli are harmless or cause relatively brief diarrhea. But a few strains, such as E. coli O157:H7, can cause severe stomach cramps, bloody diarrhea and vomiting. E. coli exhibits virulence through the K-antigens that help the bacteria to escape from the bacterial host immune response [11]. The size and composition of the K-antigen determines the degree of virulence. Staphylococcus aureus causes skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. It can also cause serious bloodstream infections, pneumonia, or bone and joint infections. Its primary mode of resistance is to produce aminoglycoside-modifying enzymes to modify aminoglycosides. Almond leaf is not a strong inhibitor of *Bacillus cereus*. The other leaf extracts were very effective in inhibiting the spoilage and pathogenic microorganisms used for the experiments (*Bacillus cereus*, *Escherichia coli* and *Salmonella typhi*). The proposed methods of involved mechanisms of action of the bioactive compounds in the leaf extracts in inhibiting spoilage and pathogenic microorganisms is shown in Figure 2.

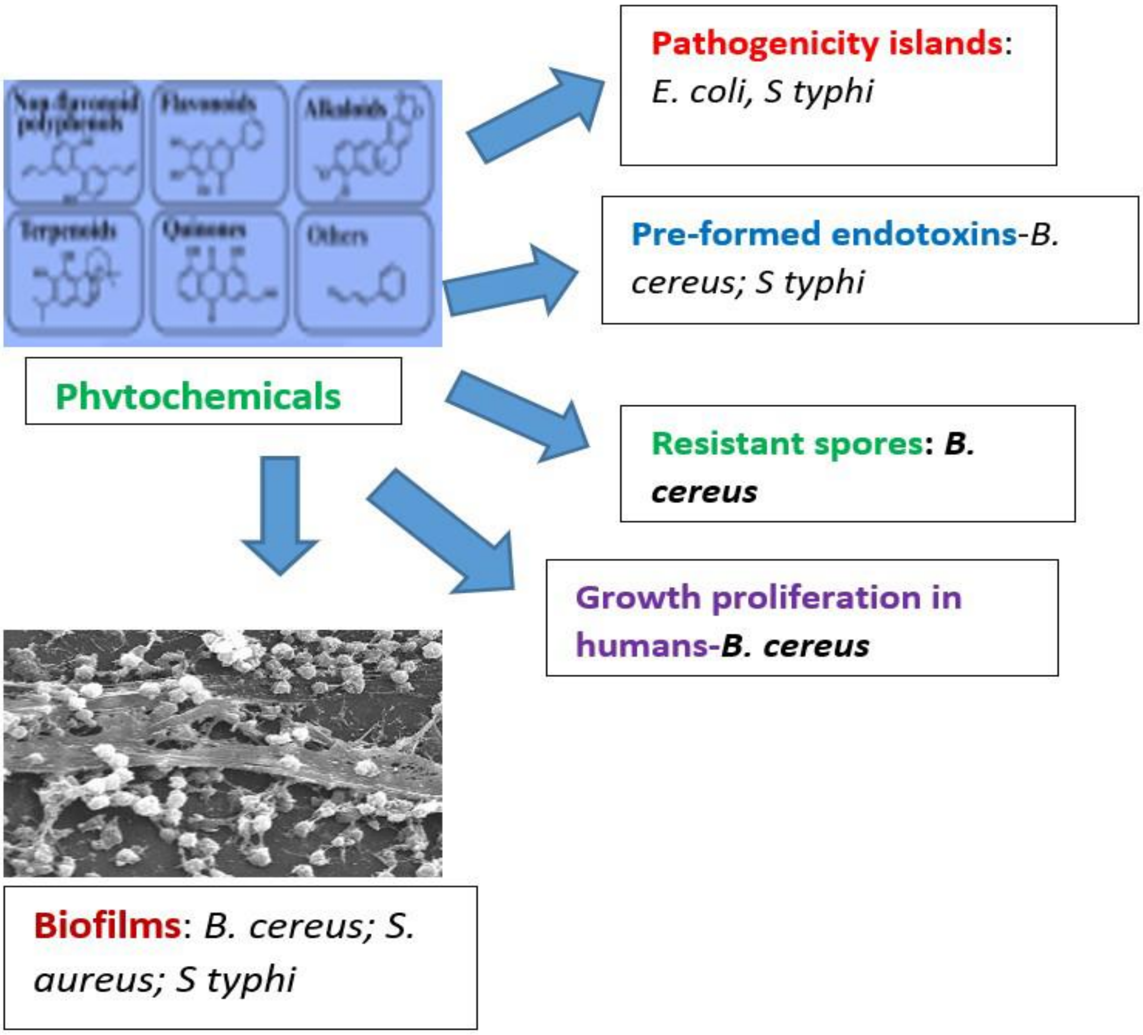


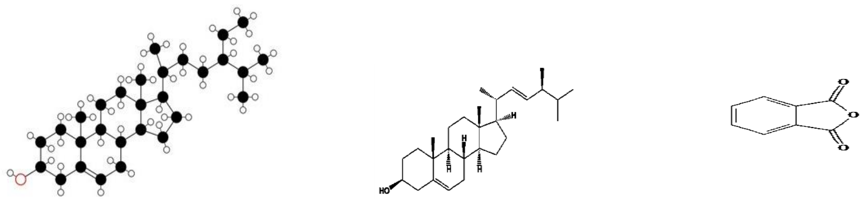
Figure 2: Proposed Mechanism of Action of The Leaf Extracts

Phytochemicals such as non-flavonoid polyphenols, flavonoids, alkaloids, terpinoids, quinones will induce pathogenicity islands in organisms such as E. coli and S. typhi; pre-formed endotoxins, and resistant spores in B. cereus (Zhou et al. [12]. Virulence in these microorganisms can be exhibited in several ways. B. cereus can proliferate in human intestines; form biofilms, endotoxins, and resistant spores [13]. S. typhi and E. coli can form pathogenicity islands [11, 14]; S, typhi and S, aureus can form biofilms to resist destruction. S. typhi can also form endotoxins. Phytochemicals and anti-microbials are the novel strategic compounds for combating virulence in pathogenic and spoilage microorganisms [15].

4.3. Volatile compounds from the leaves used for food packaging

Thirty-seven (37) compounds were identified in T daniellii leaf extract, but only those with more than 5% peak areas were recorded (see Table 2). They were alcohols, esters, fatty acids, fatty acids, amides, polysiloxane, polysteroids hydrocarbons and an ether. The Terminalia catappa (almond) leaf extract showed thirty-one (31) peaks which were mainly alcohols and alkenes. The Theobroma cacao leaf extract contained forty (40) peaks which were alkanes, alcohol, ketones, carboxylic acids and a sterol. Ojinnaka et al. [16] identified acids, esters, alcohols, furans and ketones as volatiles in fermented castor bean seeds. Ojinnaka et al. [17] also identified amines, amides, esters, and acids in raw walnuts. These results indicate that multiple functional groups contribute to the flavor and aroma of different foods and plant products.

Extract from Theobroma cacao leaves contained high amounts of Di (2-ethyhexyl) phthalate (7.81%), pentadecanoic acid, stigmasterol, gamma sitosterol, tetrapentacontane and 9,19 cyclolanost-24-en-3-ol. Volatile compounds with the highest concentrations in almond (umbrella tree) leaves were oleic acid, phthalic anhydride, 9, 10, octadecanoic acid, pentadecanoic acid and gamma sitosterol. Compounds identified in relatively high amounts in T. daniellii (African serendipity berry) leaves were: phytol (6.7%), phthalic anhydride (8.7%), Bis (2 ethylhexyl) phthalate (4.1%) and gamma sitosterol (4.8%). In effect, phthalic anhydride and related compounds and gamma sitosterol were found in all the leaves. Table 2 shows the bioactive compounds with a concentration of more than 5% found in the leaves.



Gamma sitosterol Stigmasterol Phthalic anhydride

Figure 3: Structure of Some of The Predominant Bioactive Compounds Found in The Leaves

Terminalia catappa (Almond) Leaf		
7.14	16.5	Pentadecanoic acid
7.96	17.8	9,12,-octadecadienoic acid Z, Z-
12.05	19.5	Phthalic anhydride
4.71	20.3	Gamma sitosterol
35.17	16.5	N_hexadecanoic acid; Oleic acid
12.10	17.8	Oleic acid
5.22	18.0	Octadecanoic acid
13.52	19.7	Acetic acid chlorohexadecyl ester
Theobroma cocao (cocoa) leaf		
7.43	16.5	Pentadecanoic acid
10.00	19.4	Stigmasterol
9.12	20.3	Gamma sitosterol
7.55	20.3	Gamma sitosterol
5.05	20.5	Tetrapentacontane
5.82	20.5	9,19-cyclolanost-24-en-3-ol
***Thaumatococcus daniellii* (Benn.) Benth) leaf**		
6.73	17.7	Phytol
8.74	19.5	Phthalic anhydride
4.76	20.3	Gamma sitosterol
5.98		Vitamin E

Table 2: Summary of The Concentrations of Bioactive Compounds Higher Than 5% from Each Leaf Used as a Packaging Material

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4.4. Volatile Compounds Found in The Food Products Packaged with The Leaf Packaging Materials

Compounds found in the moin-moin product wrapped with T. danielli leaves were pentadecanoic acid; 9,12-octadecanoic acid; palmidrol; phthalic anhydride; Bis (2-ethylhexyl) phthalate and gamma sitosterol (a polysterol). Ugba product wrapped in cocoa bean leaves contained high quantities of pentadecanoic acid (7.43%), stigmasterol ((10.0%), gamma sitosterol (16.6%) and phthalic anhydride (4.4%). Significant amounts of oleic acid and phthalic anhydride were found in the food product (Agidi - a corn meal concentrate) wrapped with T. daniellii leaves. Significant amounts of n-hexadecanoic acid, oleic acid, pentadecanoic acid, octadecanoic acid and acetic acid chloro-hexadecyl ester were also found in the product. Compounds transferred from the T. daniellii leaf into the cooked product (bean pudding) were stigmasterol, phthalic anhydride and sitosterol. Major compounds identified in the product (bean pudding), after packaging with T. daniella leaves were pentadecanoic acid, (7.3%), 9, 2-octadecanoic acid ((6.8%), palmidrol (5.4%), octadecanimide, N-2-hydroxyethyl (3.7%), phthalic anhydride ((6.0%), bis (2-ethylhexyl) phthalate (4.8%), and gamma sitosterol (4.4%). Figure 4 summarizes the biological activities of bioactive compounds found in the leaves especially those transferred to the food products. The major volatiles from the leaves and their health benefits are summarized in the figure 4 given below

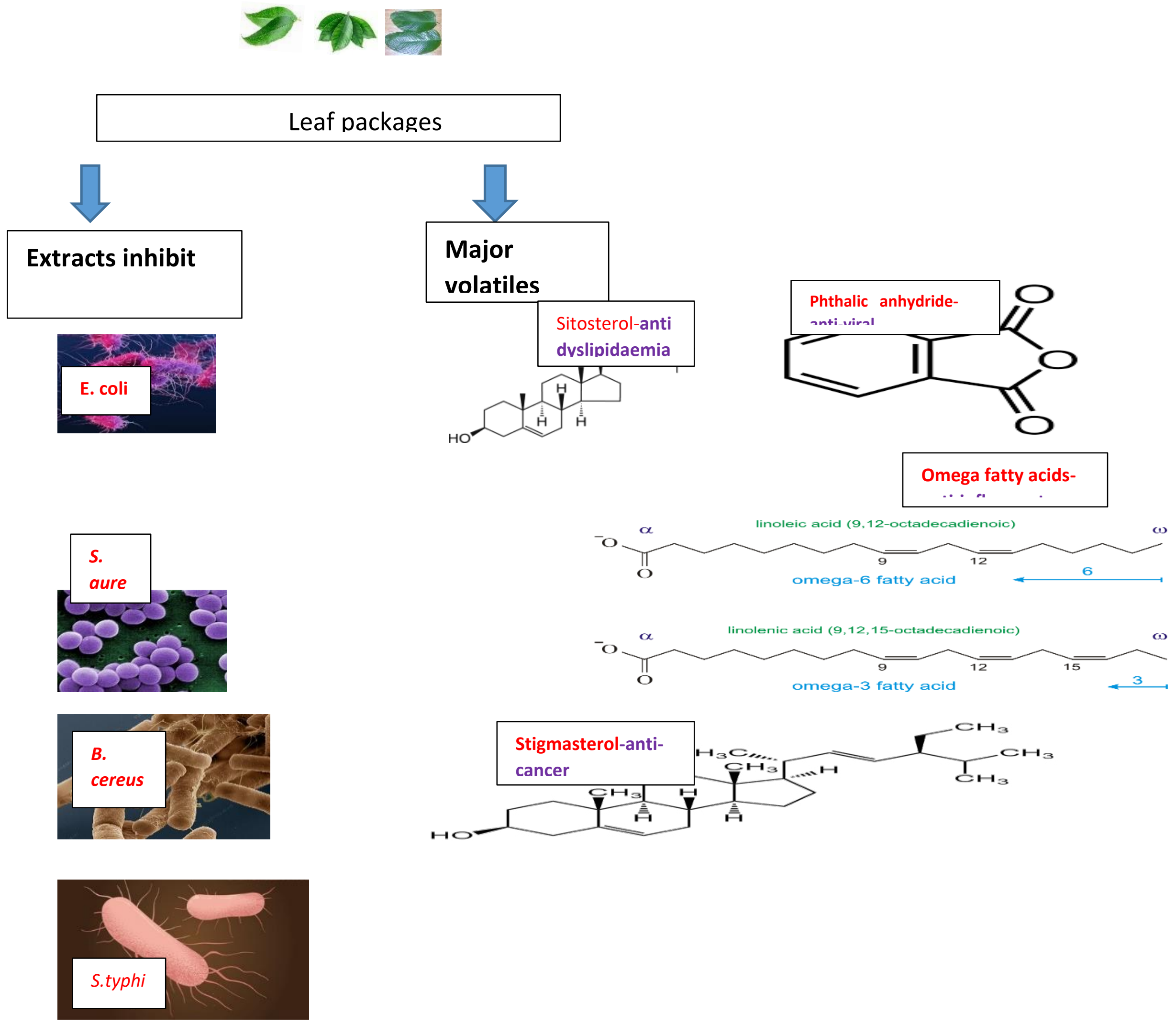

Figure 4: Public Health Benefits of Bioactive Compounds from The Leaves

Dietary stearic acid (octadecanoic acid) (C18:0), reduced LDL cholesterol, increased the levels of circulating C18:0 lipids and are associated with reduced blood pressure, improved heart function, and reduced cancer risk [18]. Beta-sitosterol lowers bad cholesterol (low-density lipoprotein (LDL) and is beneficial for managing mild to moderate benign prostatic hypertrophy (BPH). It possesses immune-modulatory and anticancer effects. Gamma-sitosterol has been observed to exhibit anti-hyperlipidemic activity, by reducing serum total cholesterol, triglycerides and VLDL [19]. Gamma-sitosterol exerts anticancer activity through inhibiting the growth, arresting the cell cycle and apoptosis of cancer cells [20]. Stigmasterol also exhibits significant anticancer properties against various tumor cell lines such as cholangiocarcinoma, hepatoma, and endometrial adenocarcinoma [21]. Phthalate showed antiviral activity

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against dengue virus, human parainfluenza virus and chikungunya. Phthalic acid has the chemical formula H2C8H4O4. Aside from the health benefit, phthalic anhydride is used to produce various chemicals, including colors, fragrances, phthalates, sugar substitutes, and many other valuable items. In lower quantities, it is also used to make the colors of anthraquinone, phenolphthalein, and porphyrin. Ledil is effective in relieving pain caused by headache, migraine, nerve pain, toothache, sore throat, period (menstrual) pains, arthritis, and muscle aches. n-Hexadecanoic acid possess anti-inflammatory properties and membrane stabilization effects [22]. Pentadecanoic acid is an odd chain saturated fatty acid that supports cardiovascular and liver health (Venn-Watson et al., [23]. Despite the health benefits of phthalic anhydride as an anti-viral agent, adverse effects were observed in workers exposed to phthalic anhydride gas on a long-term basis and included conjunctivitis, rhinitis, rhino-conjunctivitis, bronchitis, irritation of the skin and mucous membranes of the respiratory tract. Extracts from the leaves of Terminalia catappa, Theobromeo cacoa, and Thaumatococcus danielli leaves inhibited several pathogenic and spoilage organisms. Interactions between the packaging materials and the food products indicate that phthalic anhydride (and related compounds), as well as gamma sitosterol are often transferred from the leaves used as packaging materials into the food products.

5. Conclusions

The three leaf packages (used for the present study) produce extracts that can inhibit Bacillus cereus, Staphylococcus aureus, Salmonella typhi and Escherichia coli. Some of the volatiles from the leaf packages are transferred to the food products during processing. Hexadecanoic acid, pentadecanoic acid, gamma-sitosterol, and stigmasterol are some of the compounds transferred from the leaves to the food products which are beneficial to health. 10.6084/m9.figshare.31033033-Figshare 10.6084/m9.figshare.31033042

Statements and Declarations

All authors contributed to the study. Conception and design was undertaken by Philippa C Ojimelukwe. Material preparation, data collection and analysis were performed by Victoria O Kalu; Ogbonnaya I kalu; Gloria C Onyeoziri and Ifiok I Udo. The first draft of the manuscript was written by the students and revised by Philippa C Ojimelukwe. All authors approved the final manuscript.

Conflicting interests: The authors declare that they have no conflict of interest.

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