

Evaluation of the use of Terminalia catappa (Almond leaves), Theobroma cacao (cocoa leaves) and Thaumtoccoccus daniellii (miracle berry leaves) for wrapping different food products- impacts on safety, sensory properties and health benefits

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Research Article

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Abstract

The aim of this research was to evaluate the effects of traditional leaf packaging materials (*Terminalia catappa*; *Theobroma cacao* and *Thaumatococcus daniellii*) on the quality of selected food products. 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 on the food products wrapped with the leaves and was interpreted using the National Institute data base Abuja, Nigeria. The name, molecular weight and structure of the components were ascertained. The concentrations of the identified compounds were determined using area and height of peak interpretations. The water extract of almond leaves and cocoa leaves (made after blanching the cocoa leaves in boiling water for 5 min) showed antimicrobial properties against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Bacillus cereus*. Ethanol extract of *T. daniellii* leaves showed antimicrobial properties against *S. aureus*, *S. typhi*, *E. coli* but was not very effective on *B. cereus*. Corn meal concentrate (Agidi) wrapped with almond leaves contained phthalic anhydride and oleic acid transferred from the leaves (up to 90%). 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 is associated with antihyperlipidaemic activity while phthalic anhydride possess anti-viral properties. Wrapping the food products in these leaves may confer additional health benefits to man in addition to their desirable flavour properties.

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 flavour 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, thereby promoting technological advancement [6].

Functional packaging can be used to enhance the status of specialized products to meet the nutritional 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. Plant leaves have been used to package food all over the world. 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.

2.1.1. Testing for the antimicrobial properties of leaf extracts

In line with the ICFMS [10] method, the microbial disc technique was used. In this technique, the leaf extract were prepared in three concentrations which were 100% (10^0), 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 °C, poured into sterile petri-dishes and allowed to solidify for about 45-60 minutes. 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 diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were afterwards incubated for 18-24 h at 37 °C. Using Whatman NO 1 Filter paper. Microbial testing discs were prepared and using 0.5ml of each of the extract concentrations the disc were 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 °C for 24-48 hours respectively except the PDA plates which were incubated at a room temperature of 20-25 °C for 2-3 days because the growth performance of fungi is best at ambient temperature and very poor at temperatures above ambient (above 30°C).

2.1.2. 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 a sterile forcep, 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 centre 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 extracts 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.

2.1.3. Determination of volatile compound

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 µl 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.

2.1.4. 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.

3. Results and Discussion

3.1. Inhibitory effects of leaf extracts on spoilage and pathogenic microorganisms

The leaves used to wrap the food products are shown in Figure 3. Table 1 shows the inhibitory effects of these leaf extracts on *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*.

Table 1. Inhibitory potentials of plant leaf extracts on selected spoilage and pathogenic microorganisms

Plant leaf Extract	Microorganisms	Activity	Zone of inhibition (mm)	Minimum inhibitory concentration (MIC)
Cocoa leaf extract	<i>Bacillus cereus</i>			
10^0		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}		+	30	
	<i>Escherichia coli</i>			
10^0		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}		+	30	
	<i>Staphylococcus aureus</i>			
10^0		+++	120	10^{-2}
10^{-1}		++	60	
10^{-2}		+	30	
	<i>Salmonella typhi</i>			
10^0		+++	120	
10^{-1}		++	60	
10^{-2}		+	30	
Almond Leaf Extract	<i>Bacillus cereus</i>			
10^0		+	30	10^{-0}
10^{-1}		-	0	
10^{-2}		-	0	
	<i>Escherichia coli</i>			
10^0		+++	120	10^{-2}

10^{-1}	++	60	
10^{-2}	+	30	
<i>Staphylococcus aureus</i>			
10^0	+++	120	10^{-2}
10^{-1}	++	60	
10^{-2}	+	30	
<i>Salmonella typhi</i>			
10^0	+++	120	10^{-2}
10^{-1}	++	60	
10^{-2}	+	30	
<i>T. danliella</i> leaf extract			
<i>Bacillus cereus</i>			
10^0	+++	120	10^{-2}
10^{-1}	++	60	
10^{-2}	+	30	
<i>Escherichia coli</i>			
	+++	120	10^{-2}
	++	60	
	+	30	
<i>Staphylococcus aureus</i>			
10^0	+++	120	10^{-2}
10^{-1}	++	60	
10^{-2}	+	30	
<i>Salmonella typhi</i>			
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; 10^0 = 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

3.2. 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*).

Virulence in these microorganisms can be exhibited in several ways. *B. cereus* can proliferate in human intestines; form biofilms, endotoxins, and resistant spores [12] *S. typhi* and *E. coli* can form pathogenicity islands [11, 13]; *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 [14].

3.3. 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 (42) peaks which were alkanes, alcohol, ketones, carboxylic acids and a sterol. Ojinnaka *et al.* [15] identified acids, esters, alcohols, furans and ketones as volatiles in fermented castor

bean seeds. Ojinnaka *et al.* [16] 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. Summary of the concentrations of bioactive compounds higher than 5% from each leaf used as a packaging material

<i>Terminalia catappa</i> (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 acidOleic acid
12.10	17.8	Oleic acid
5.22	18.0	Octadecanoic acid
13.52	19.7	Acetic acid chlorohexadecyl ester
<i>Theobroma cacao</i> (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
<i>Thaumatococcus daniellii</i> (Benn.) Benth) leaf		
6.73	17.7	Phytol
8.74	19.5	Phthalic anhydride
4.76	20.3	Gamma sitosterol
5.98		Vitamin E

3.4. Volatile compounds found in the food products packaged with the leaf packaging materials

Compounds found in the moin-moin product wrapped with *T. daniellii* 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%).

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 [17]. 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-hyperlipidaemic activity, by reducing serum total cholesterol, triglycerides and VLDL [18]. Gamma-sitosterol exerts anticancer activity through inhibiting the growth, arresting the cell cycle and apoptosis of cancer cells [19]. Stigmasterol also exhibits significant anticancer properties against various tumor cell lines such as cholangiocarcinoma, hepatoma, endometrial adenocarcinoma [20], Phthalate showed antiviral activity against dengue virus, human parainfluenza virus and chikungunya. Phthalic acid has the chemical formula $\text{H}_2\text{C}_8\text{H}_4\text{O}_4$. Aside from the health benefit, phthalic anhydride is used to produce various chemicals, including colours, fragrances, phthalates, sugar substitutes, and many other valuable items. In lower quantities, it is also used to make the colours-

anthraquinone, phenolphthalein, and porphyrin. Ledol 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 [21]. Pentadecanoic acid is an odd chain saturated fatty acid that supports cardiovascular and liver health [22]. 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.

4. Conclusion

Extracts from the leaves of *Terminalia catappa*, *Theobroma cacao*, and *Thaumatococcus danielli* leaves inhibited *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. *T. catappa* did not show strong inhibition to *B. cereus*. Findings from the results of 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. Care must be taken to ensure that the leaves used for packaging are not contaminated, so that they do not transmit pathogenic and spoilage microorganisms into the food products.

Declarations

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

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Figures



T danielli



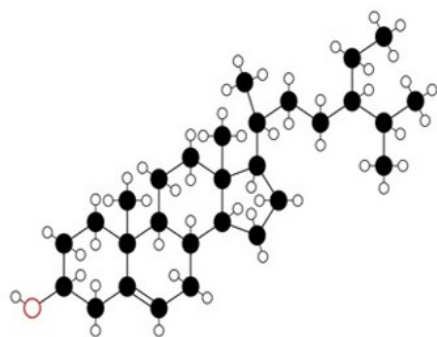
T. catappa



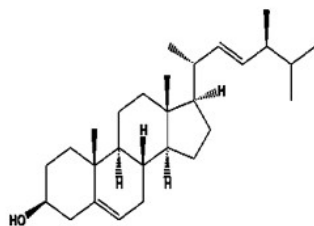
T cacao

Figure 1

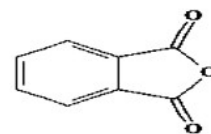
(*Thaumatooccus danielli*; *Terminalia catappa*; *Thebroma cacao*)



Gamma sitosterol



Stigmasterol



Phthalic anhydride

Figure 2

Legend not included with this version

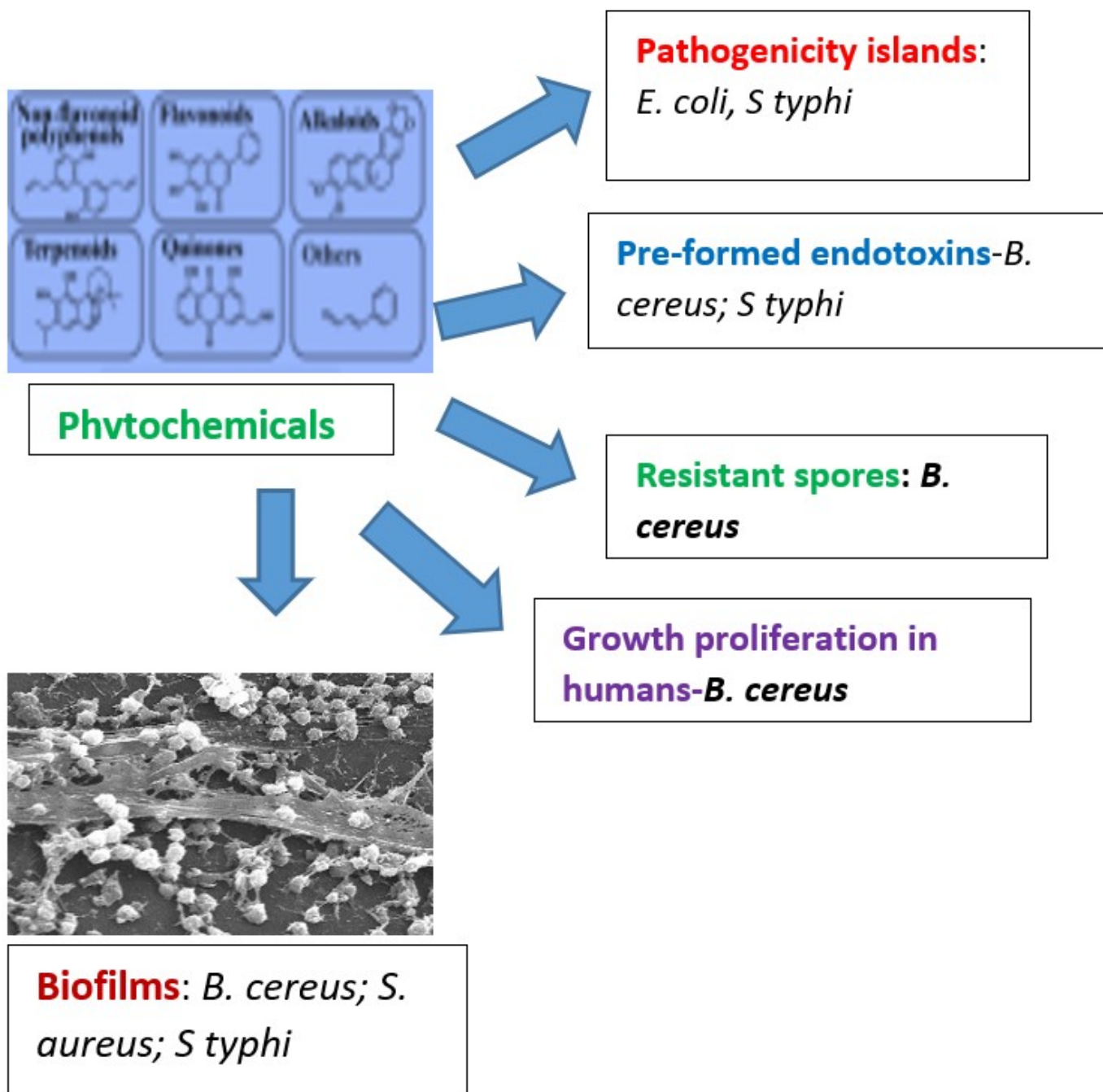


Figure 3

Proposed mode of inhibition of pathogenic and spoilage microorganisms

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