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Research article

Phytochemical screening and antibacterial activities of *Tectona grandis* L. f. (Teak) leaves on microorganisms isolated from decayed food samples

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Abstract: Bacteria were isolated from decayed food samples (tomatoes, cooked beans and rice) collected from Afe Babalola University, Ado Ekiti (ABUAD) cafeteria and characterized. Some of these isolated microorganisms could pose serious harm to humans including animals and they are normally treated with commercial antibiotics. However, the majority of bacteria are resistant to many antibiotics therefore, the use of plant extracts with therapeutic potential against resistant bacteria is necessary. In this investigation, eight bacteria were isolated from decayed food samples. The bacterial isolates were identified as *Bacillus cereus* and *B. siamensis* from rice sample; *Klebsiella oxytoxa*, *Salimicrobium halophilium* and *Norcardia brasiliensis* from beans sample; *Bacillus subtilis*, *Enterobacter taylore*, and *Brevibacillus agri* from tomatoes. The leaf samples of *Tectona grandis* were screened qualitatively and quantitatively for the phytochemicals while the crude methanol and chloroform extracts were used as antimicrobial agents against the isolated microorganisms. Alkaloids, carotenoids and tannins were present in large amount. The bacterial isolates were more susceptible to commercial antibiotics than that of methanol extracts of *T. grandis*. The methanol extracts of *T. grandis* have a higher antimicrobial activity than the chloroform extracts.

Keywords: Chloroform extracts - Food samples - Methanol extracts - Microorganisms - *Tectona grandis*.

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INTRODUCTION

Food decay is any undesirable change in food that makes the food to lose its aesthetic value and becomes unacceptable to humans for consumption. Food is also said to be decayed when the original nutritional value, texture and flavor are altered through microbial activities. Various factors could be responsible for the deterioration of various foods (Istifanus *et al.* 2014). Consumption of this decayed or contaminated food results in food borne disease such as diarrhoea, gastroenteritis, respiratory infections and meningitis, if ingested (Mead *et al.* 1999, Humphrey *et al.* 2007, Barth *et al.* 2009). Also, toxins from these microorganisms can be found in contaminated food products and are pathogenic leading to disease (Edwin *et al.* 2013). Although, bacteria are the main and important source of food spoilage, but other microbes such as fungi, viruses and protozoa have also been implicated (Kumar *et al.* 2011). The most common pathogens causing serious infection in food include *Staphylococcus aureus*, *Pseudomonas* sp., *Streptococcus* sp., *Proteus* sp., *Clostridium* sp. and Coliforms (Lawrence *et al.* 1999). High pH (4.9–6.5), water and nutrient contents enhance microbial growth such as bacteria and fungi, which degrade the nutrients through enzymes production (Trias *et al.* 2008, Matthew 2011, Ogunbanwo *et al.* 2014). These also heighten spoilage susceptibility thereby reducing the nutritional and market values (Bello *et al.* 2016). Most of the pathogenic microorganisms causing diseases have developed resistance to modern antibiotics (Sharma *et al.* 2012). Bacteria generally have the genetic ability to transmit diseases and acquire resistance to drugs used as therapeutic agents (Cohen *et al.* 1992). Therefore, there is needed to look for new antimicrobials of plant origin.

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Medicinal plants are gaining popularity in usage due to a large number of people in search of health remedies with little or no side effect which is the problem of most chemically synthesized drugs (Prance 1991, Bajpai *et al.* 2016). Considerable attention is presently given to the use of eco-friendly and bio-friendly products from plant origin for the prevention and cure of human and animal health challenges (diseases) (Gijtenbeek *et al*. 1999, Johnson & William 2002). Plants based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potentials (Evans & Turnbull 2004). The majority of bacteria are resistant to many antibiotics therefore, the use of plant extracts against resistant bacteria leads to new choice for the treatment of infectious diseases (Purushotham *et al*. 2010).

Tectona grandis L. f. (Teak) is a tropical tree species distributed naturally in countries including India, Myanmar, Thailand, Myanmar, Nigeria and other tropical countries including Indonesia (Mahesh *et al.* 2016). Teak is one of the world's premier hardwood tree species, highly famous for its quality, profile and durability of timber (Sumthong *et al*. 2008). The generic name of *T. grandis* comes from 'Tekka', which is the Malabar name while the specific name, '*grandis*' is a Latin word for 'large' or 'great'. *Tectona grandis* is a large, deciduous tree reaching over 30 m in height in favourable conditions (Orwa *et al*. 2009). "Fruit is a drupe with 4 chambers; round, hard and woody, enclosed in an inflated, bladder-like covering; pale green at first, then brown at maturity" (Orwa *et al*. 2009). It occurs naturally in various types of tropical deciduous forests. In seasonal climates, *T. grandis* is deciduous while trees grown in non-seasonal climates are semi-deciduous. It is often a dominant member of a mixed deciduous forest, where its main associates are *Xylia* spp., *Afzelia xylocarpa* (Kurz) Craib, *Terminalia* spp. and *Lagerstroemia* spp. *Tectona grandis* generally occurs scattered but can form almost pure stands under favourable conditions. Young plants show a remarkable capability to recover after fire (Orwa *et al*. 2009).

Diverse anti-microbial and substances that can serve as insect control agents can be produced by higher tropical plants (Nakamura *et al*. 1991, Downum *et al*. 1993, Lis-Balchin *et al*. 1996). Substances such as flavonoids, alkaloids, terpenoids are the secondary metabolites produced by the plants as a chemical defense against pests and diseases attacks. It is estimated only 10% of these tropical plants have been investigated for their pesticidal activity. Teak is one of these plants which produce secondary metabolic products containing phenolic compounds. Astiti *et al.* (1998) found that the water extract of teak leaf obviously inhibited the growth of *Monilia* species, the causative agent of wood decay. The sporulation of *Alternaria cajani* and *Helminthosporium* sp. was inhibited by the *T. grandis* leaf methanol crude extract at concentration of 5 $mg.ml^{-1}$ (Shalini & Srivastava 2008). The frontal leaves of the plant are widely used in folklore for the treatment of various kinds of wounds, especially burn wounds. They are also useful to treat haemostatic, depurative, anti-inflammatory and vulnerary and also useful in inflammation, leprosy, skin disease, pruritus, stomatitis, indolent ulcer, haemorrhages and haemopstysis (Purushotham *et al.* 2010). The presence of high amounts of tannins in the *T. grandis* leaves may be responsible for the pro-healing action of the extract and its antioxidant property is attributed to it (Mrityunjoy *et al.* 2007). All these studies clearly showed the antibacterial potential of *T. grandis*, thus the present study was designed to find out the effect of its leaves on the microorganisms isolated from decayed food samples.

MATERIALS AND METHODS

Collection of food samples

Food samples (fresh tomatoes, cooked beans, cooked rice and fresh pepper) were collected from Afe Babalola University, Ado-Ekiti (ABUAD) cafeteria/kitchen environment. These samples were allowed to decay in ABUAD Microbiology Department laboratory.

Collection of plant sample

The leaves of *Tectona grandis* L. f. were obtained from ABUAD surroundings and were identified by Dr. (Mrs.) O. T. Ogunmefun, a botanist in the Department of Biological Sciences of Afe Babalola University Ado - Ekiti, Ekiti State, Nigeria.

Preparation of extracts

The collected plant materials were air dried at room temperature, ground with blender after which dry mass of 887.2 g of the leaf sample was obtained (Purushotham & Sankar 2013). A 350 g each of powdered leaf sample was soaked in 700 ml of chloroform and methanol separately using cold extraction method for 72 hours.

The extracts were separated from the solvents using a rotary evaporator. The extracts were concentrated on water bath at a low temperature of 40°C (Ajaiyeoba *et al.* 2001, Ogbole *et al.* 2013).

Phytochemical screening of Tectona grandis

The powdered leaves of *T. grandis* were subjected to both qualitative and quantitative phytochemical tests for plant secondary metabolites namely alkaloids, anthraquinones, tannins, saponins, cardiac glycosides, flavonoids, carotenoids and phenols according to the method described by Harborne (1998) and Trease & Evans (2004).

Isolation, characterization and identification of microbial species from the decayed food samples

Five-fold serial dilution was carried out on the food samples. An aliquot (1 ml) of the fifth dilution was pour plated on nutrient agar and then incubated at 37°C for 24 hours. Colonies of bacterial isolates were selected from each plate respectively and purified by sub-culturing on nutrient agar plates. Bacterial isolates were preserved in slants at 4°C for temporary storage and were then identified both morphologically and biochemically (Prescott *et al*. 2002).

Determination of the antibacterial activities of Tectona grandis using agar well diffusion technique

A sterile swab stick was placed into the broth bacterial culture corresponding to MacFarland standard of a specific organism and then spread on already prepared sterile nutrient agar plate. This was repeated for all the isolates in duplicates. The plates were allowed to dry for approximately 5 minutes. The wells were then filled with dilutions of 50 $mg.ml^{-1}$, 100 $mg.ml^{-1}$, 150 $mg.ml^{-1}$, 200 $mg.ml^{-1}$, 250 $mg.ml^{-1}$, 300 $mg.ml^{-1}$, 350 $mg.ml^{-1}$ and 400 $mg.ml^{-1}$ with *T. grandis* extract reconstituted with 10% Dimethyl sulphoxide (DMSO) and sterilized by 0.45 µm membrane filter. The plates were incubated 37°C for 24 hours. The diameters of the inhibitory zones were measured in millimeters. The DMSO was used as a negative control while streptomycin was used as the positive control.

RESULTS

In this study, seven bacterial species were obtained from all the decayed food samples based on their morphological and biochemical characteristics. Five of the isolates were Gram positive while the remaining two isolates were Gram negative. The isolates were identified as *Bacillus cereus, B. subtilis, B. siamensis, Brevibacillus agri, Salimicrobium halophilum, Klebsiella oxytoxa, Norcadia brasiliensis* and *Enterobacter taylore*. The frequency of occurrence of the isolate is presented in table 1.

Table 1. Frequency of occurrence of bacterial isolates from decayed food samples.

Bacterial isolates	Food samples		
	Beans	Rice	Tomatoes
Bacillus cereus	-	+	-
Bacillus siamensis	-	+	+
Bacillus subtilis	-	-	+
Klebsiella oxytoxa	+	-	+
Salimicrobium halophilum	+	-	-
Enterobacter taylore	-	-	+
Norcadia brasiliensis	+	-	-
Brevibacillus agri	-	-	+

Note: +, Present; -, Absent.

Saponins, alkaloids, tannins among others were present while cardiac glycoside was not observed in the qualitative screening. However, the quantitative analysis showed that 20 mg per 100 g of cardiac glycoside was present. Alkaloids were most abundant followed by tannins and carotenoids respectively. The detail results of the qualitative and quantitative phytochemicals of *T. grandis* are presented in tables 2 and 3.

The leaf extracts obtained with chloroform and methanol were used as antibacterial agents against the bacterial isolates. The results showed that the extracts exhibited weak antibacterial activities on the isolates (Table 4). The antibacterial activities of streptomycin used as positive control ranged from 11.00–30.00 mm respectively and were without a doubt effective against food pathogens than the extract. All the bacterial isolates were resistant to DMSO used as negative control (Fig. 1). At concentrations of 50, 100, 150 and 200 $mg.ml^{-1}$, the extracts were not effective but at concentrations of 250, 300, 350 and 400 $mg.ml^{-1}$ there were clear zones of inhibition (Table 4).

Table 2. Qualitative phytochemical analysis of *Tectona grandis* leaves.

Parameter	Observation
Alkaloids	+++
Flavonoids	++
Tannins	+++
Saponins	++
Cardiac Glycosides	-
Total Phenolics	++
Anthraquinones	+
Carotenoids	++

Note: +++, Most abundant; ++, Abundant; +, Present; -, Absent.

Table 3. Quantitative phytochemical analysis of *Tectona grandis* leaves.

Phytochemical constituents	Amount of Phytochemical constituents
Alkaloids (mg per 100 g)	1778.30
Flavonoids (mg per 100 g)	666.60
Tannins (mg per 100 g)	1583.00
Saponins (mg per 100 g)	406.60
Cardiac Glycosides (mg per 100 g)	20.00
Total Phenolics (GAE per g)	69.50
Carotenoids (µg per 100 g)	1268.33

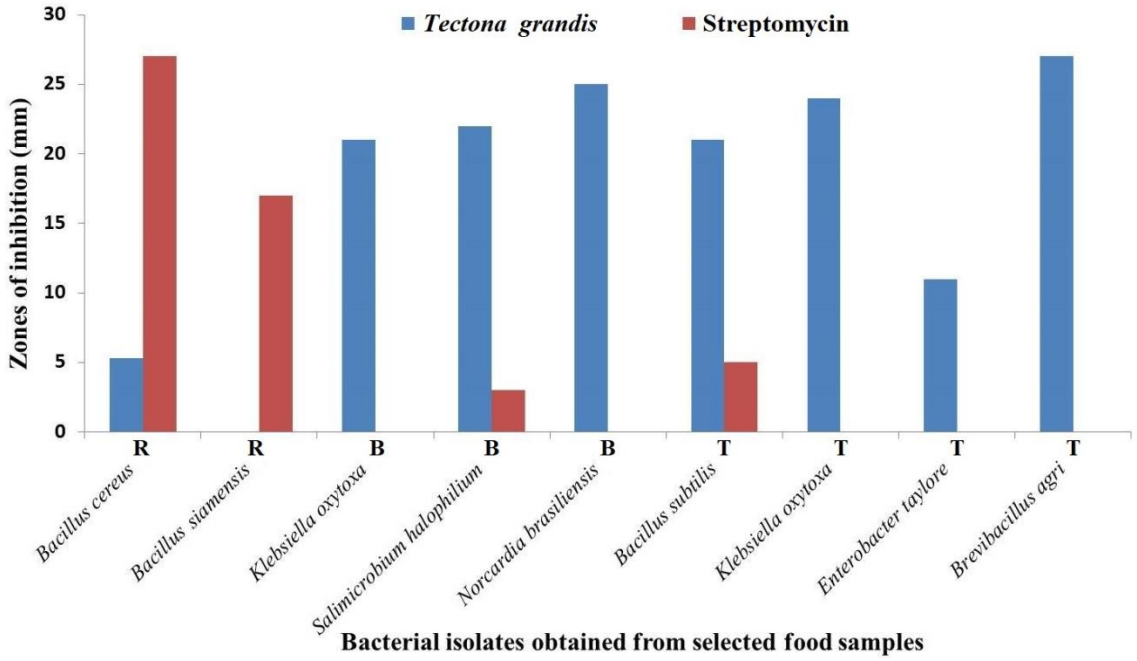

Figure 1. Comparative antibacterial activities of methanol extract of *Tectona grandis* and Streptomycin at 37°C on nutrient agar after 24 hours of incubation. [R, Rice; B, Beans; T, Tomatoes]

DISCUSSIONS

Different spoilage bacteria including fungi grow well at room temperature. A large number of microorganisms and their waste products cause the objectionable changes in odor, taste and texture in food samples (Kumar *et al.* 2011). The presence of these bacterial isolates may not be unconnected with the high moisture contents of the food substances which aided the spoilage as opined by Akharaiyi & Adeyanju (2016). *Bacillus cereus* has been associated with the production of toxins, diarrheal and emetic in food, which causes food poisoning. It is found in dust, soil and raw food and can survive under normal cooking conditions as produces heat resistant spore (Rajkowski & Bennett 2003). Bello *et al*. (2016) observed the prominence of *B. subtilis* in spoilage of tomato fruits. Ghosh (2009) also suggested that the prevalence of microbial contamination could be aggravated by poor sanitation including cross-contamination. Biological contaminant of bacterial origin represents a major cause of foodborne illnesses (Edema *et al.* 2005). The presence of *Bacillus subtilis* has been correlated with food poisoning. *Klebsiella* spp. and *Enterobacter* spp. call for concern as these organisms

are frequently associated with poor sanitary practices and could indicate the danger of possible food borne infection (Oranusi *et al*. 2007, Eni *et al*. 2010). *Klebsiella* spp. has been isolated in food such as fruits and vegetables, meat, milk and dairy products, salads, and drinking water, coming from the general environments of soil, dust, air and water and from social environments.

Table 4. Antibacterial activity of methanol extract of *Tectona grandis* leaves.

Bacterial isolates	**Concentration of crude extract (mg.ml^{-1})**							
	50	100	150	200	250	300	350	400
P3B3	R	R	R	R	10.00	10.00	11.00	11.50
P2B1	R	R	13.00	13.00	15.00	15.00	18.00	18.00
P3B3	R	R	R	2.00	4.00	6.00	11.00	12.00
P2B2	R	R	R	R	R	R	14.00	14.00
P1B1	R	R	R	R	R	R	R	R
RIB1	R	R	R	16.00	11.00	15.00	15.00	15.50
RB1	R	R	R	R	R	R	R	R
R1B2	R	R	R	R	R	R	R	R
R1B1a	R	R	R	R	R	R	R	R
T1B2	R	R	10.50	13.00	14.50	1.50	14.00	15.00
T3B1	R	R	R	12.00	12.50	13.50	15.00	18.50
T2B1	1.00	2.00	2.00	4.00	4.00	5.00	6.00	7.00
T1B1	R	R	3.00	4.00	4.00	6.00	6.00	8.00
T3B2	R	R	2.00	3.00	5.00	5.00	6.00	10.00

Note: PB, Isolates obtained from beans; **RB**, Isolates obtained from rice; **TB**, Isolates obtained from tomatoes; **B**, Bacteria; **R**, Resistance.

Higher tropical plants can produce diverse antimicrobial and anti-insect substances (Nakamura *et al*. 1991, Downum *et al*. 1993, Lis-Balchin *et al*. 1996). Substances such as flavonoids, alkaloids and terpenoids are secondary metabolites produced by plants as a chemical defence against pests and diseases' attacks. Tannins are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda 2003); they are referred to as natural antibiotics from plants.

The water extract of *T. grandis* leaf conspicuously inhibited the growth of *Monilia* sp., which is a causal organism attributing in wood decay (Astiti 1998). The methanol crude extract of *T. grandis* leaves at 5 mg.ml^{-1} concentration inhibited the sporulation of *Alternaria cajani* and *Helminthosporium* sp. by 86.6 and 90.0% respectively (Shalini & Srivastava 2008).

CONCLUSION

The methanol extract of *Tectona grandis* leaf significantly inhibited bacterial growth. Chloroform extract was least effective on bacterial isolates as compared to methanol extracts. Teak extracts can be considered as one of the alternatives to chemical food preservatives and for controlling food decay caused by these isolates. A further study is needed to isolate and identify the active compounds that are responsible for the antimicrobial potency of *T. grandis.*

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