

Antibacterial Properties and Phytochemicals Screening of *Tectona grandis* (Teak) Leaf Extracts against Bacteria Implicated from Upper Respiratory Tract Infections

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Received: 23 September 2022; **Accepted:** 07 October 2022; **Published:** 07 November 2022

Citation: O. O. Daramola, S. God'swealth U., Unwana E.O., O.M. Olugbenga1, (2022). Antibacterial Properties and Phytochemicals Screening of *Tectona grandis* (Teak) Leaf Extracts against Some Clinical Isolates Causative Agents of Upper Respiratory Tract Infections. *Journal of Microbes and Research*. 1(1). DOI: 10.58489/2836-2187/004

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Abstract

Upper respiratory tract infections (URTIs) commonly befalls both children and adults and is a major cause of mild morbidity. URTIs ranges from mild self-limiting diseases such as common cold to serious life-threatening illnesses such as epiglottitis. The present study was aimed to evaluate the antibacterial activities of *Tectona grandis* (teak) leaves extracts on some pathogenic microorganisms isolated from clinical samples causing URTIs. The antibacterial activities were assayed using the agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated using standard microbiological techniques. Phytochemical and mineral compositions of all the extracts were determined. Appreciable quantities of phytochemical such as saponins, tannins, flavonoids, glycosides, anthraquinone and alkaloids were present in both extracts of the plants with flavonoids having a higher percentage. Minerals such as K, Ca, Fe, Na and Fe were presented in both solvents. Heavy metals like Pb, Cd and as were presented in significant quantities in both extracts, however, high amount of the heavy metal composition was observed in the ethanol extract. The plant extracts exhibited varying degrees of concentration-based antibacterial activities against bacteria implicated from URTIs. They showed a high significant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Conclusively, the plant extracts contained several bioactive compounds (phytochemical), thereby validating the use of this plants for therapeutic tenacities. Also, the antibacterial activity of the leaf extracts against the test organisms also supports its usage in traditional medicine practice.

Keywords: antibacterial, upper respiratory tract infections, *Tectona grandis*, antibiotics resistance.

Introduction

Upper respiratory tract infections (URTIs) are caused by mainly viruses (human rhinovirus (hRV), coronavirus, parainfluenza viruses (PIVs), adenovirus (ADV), human metapneumovirus (hMPV), influenza, enterovirus, bocavirus and respiratory syncytial virus (RSV)) and some bacteria, though present with almost indistinguishable clinical symptoms (Heikkinen and Jarvinen, 2003, Allander, 2008; Caliendo, 2011; Harris et al., 2016; Pokorski, 2016). In most cases, it spreads from person-to-person, when touching the secretions by hand or directly

inhaling the respiratory droplets. Bacterial infections could be a prime cause of URTIs, but they may also be due to superinfection of a primarily viral infection (Turner, 2007). Risk factors for the development of upper respiratory tract infections are close contact with infected person, most likely close contact of children who attend the kindergarten or school, travelers with exposure to numerous individuals, smoking which may alter mucosal resistance, anatomic changes of respiratory tract, and nasal polyposis (Heymann, 2014). URTIs causes variety of patient diseases such as acute bronchitis, the

common cold, influenza, and respiratory distress syndromes (Thomas and Bomar, 2022). It has a high cost to society, being responsible for absenteeism from school and work, unnecessary medical care and is occasionally associated with serious sequelae (Cotton et al., 2008). It affects the nose, nasal cavity, pharynx, and larynx with subglottic area of trachea (Green, 2006). Lack of timely diagnosis by using rapid and accurate tests had contributed to overuse and abuse of antibiotics around the world (Laxminarayan et al., 2013). In developing nations, antibiotics abuse is very common due to a culture of self-medication and over-prescription by clinicians (Li et al., 2016). Antibiotics are mainly prescribed empirically, not based on microbiological investigation (Yezli and Li, 2012; Zeng et al., 2017).

Antibiotic resistance has become a global concern (Westh et al., 2004) as the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens (Bandow et al., 2003). 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). Antimicrobials of plant origin have enormous therapeutic potentials (Evans and Turnbull, 2004). Plants are rich source of phytochemicals which are responsible for the therapeutic potentials, therefore, plants are used for making different drugs having medicinal property. 25% of the medicinal drugs prepared in the developed countries are based on the plants and their derivatives (Moses et al., 2019). Therefore, this has led to the screening of several medicinal plants for possible antimicrobial activity (Iwu et al., 1999).

Tectona grandis Linn, belongs to the family of Lamiaceae. It can be found in India, Pakistan, Bangladesh, Burma, Indonesia, Thailand, Nigeria, Philippines, China and Malaysia. It is widely cultivated in other tropical areas, as its wood is moderately hard, easily worked, and extremely durable (Uzama and Okolo, 2017). The leaves and other parts of the tree are used as diuretics, laxative, sedative, expectorant, anthelmintic, anti-inflammatory, antibacterial, cytotoxic, anti-anaemic, haemostatic, depurative, vulnerary, leprosy, burn wounds, skin disease, pruritus, haemoptysis, antiulcer, antiviral, menstrual disorders, hemorrhages, sore throat, dyspepsia and burning of stomach caused by bile over flow, vermifuge, strengthening of sight, treatment of pile, dysentery, antidiabetic, antioxidant, antipyretic, as dye and incense (Mrityunjoy et al., 2007; Mahesh and Vijay, 2009; Purushotham et al., 2010; Bitchagno et

al., 2015; Mahesh et al., 2016). Previous phytochemical investigations have led to the isolation of the triterpenoids, flavonoids (Ragasa et al., 2008a), chromomeric acid derivatives (Aguinaldo et al., 1997; Ragasa et al., 2008b), anthroquinones (Sumthong et al., 2006; Sumthong et al., 2008; Kopa et al., 2014), naphthoquinones (Pradeep and Pahup, 2004; Lacret et al., 2011), anthraquinone-naphthoquinones (Aguinaldo et al., 1993; Lacret et al., 2011), apocarotenoids (Macias et al., 2008) and lignans (Lacret et al., 2012). This study is designed to evaluate the antibacterial activities of ethanol and ethyl acetate extracts of *Tectona grandis* leaves on against bacteria implicated from upper respiratory tract infections through determining the Minimum inhibitory concentration (MIC) and Minimum bacterial concentration (MBC) of the plant extracts.

Materials And Methods

Equipment, Chemicals and Reagents

Materials used in laboratory included beaker, measuring cylinder, porcelain dish, spatula, conical flask, retort stand, incubator, petri-dish, autoclave, cork borer, sterile loop, cotton wool, spirit lamp, test tubes and racks, aluminum foil, hand gloves, disposable pipette, filter paper (Whatman No. 1), electric blender, atomic absorption spectrophotometer (AAS), Erlenmeyer flask, Pyrex volumetric flask, analytical and mechanical weighing balance. All chemicals and reagents used in this work were of analytical grade and they included ferric chloride solution, Fehling's solution A and B, hydrochloric acid (concentrated and dilute), sodium bicarbonate, sodium hydroxide and dilute ammonia solution. Tetraoxosulphate (vi) acid, glacial acetic acid, lead acetate, 10 % alcoholic solution of naphthol, Mayer's reagent, Dragendorff's reagent, picric acid solution (Hager's reagent), Wagner's reagent, ciprofloxacin (standard drug), nutrient agar, perchloric-nitric acid, concentrated HNO₃, concentrated Sulphuric acid, ethanol and ethyl acetate were also included among others.

Collection, Identification and Extraction of Plant Materials

Fresh leaves of *Tectona grandis* were collected from the vicinity of D. S. Adegbenro ICT Polytechnic, Itori, Ewekoro Local Government Area, Ogun State, Nigeria. The identification and authentication of the plant materials was done at Forest Research of Nigeria, Jericho Hill, Ibadan, Oyo State, with the voucher number of FHI 113357. The collected sample was air dried at room temperature for twenty-one (21) days. The samples were ground into powdery state using an electric blender. 200 g of the ground sample

of the leaf was weighed and dissolved in 500 mL of ethanol, again 200 g of the ground sample of the leaf was weighed and dissolved in 500 mL of ethyl acetate. They were kept in the refrigerator for 72 hours. The extract was filtered using a chess cloth and Whatman filter paper No. 1 (125 cm), to obtain filtrates of the respective solvents of ethanol and ethyl acetate, which were then used for the study.

Collections and Maintenance of Test Organisms

The test organisms used for this study were all clinical isolates from the Department of Medical Microbiology and Parasitology, Sacred Heart Hospital, Lantoro, Abeokuta, Ogun State. The isolates include; *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The organisms were collected on a sterile agar slant and incubated at 37°C for 24 hours.

Preparation of Agar

Agar was prepared according to manufacturer instruction and the agar used was nutrient agar for the analysis. Seven (7) grams of nutrient agar was weighed and dissolved in 250 ml of distilled water, mixed and was autoclaved at 121°C for 15 minutes.

Antimicrobial Assay (Agar Well Diffusion Method) Antimicrobial activities of ethyl acetate and ethanol extracts of *Tectona grandis* L. leaf were evaluated by the agar well diffusion method (Bauer et al., 1996) using ciprofloxacin as positive control. The microbial culture was adjusted to 0.5 McFarland turbidity standards. The plate was flooded with 1 ml each of the standardized test organism, swirled and excess inoculum was carefully decanted. A sterile cork borer was used to make wells (6 mm in diameter) on the agar plates. Aliquots 0.2 ml of dilution were applied on each well in the culture plates previously inoculated with the test organisms. The holes were filled with the plant extract. Each well was labeled approximately; control experiment was also carried out where the hole was filled with ciprofloxacin as positive control for bacteria. However, each extract was tested in triplicates. These were then left on the bench for 1 h for proper diffusion of the nanoparticles (NCCLS, 1990). Thereafter the plates were incubated at 37°C for 24 h for bacteria. Antimicrobial activities were determined by measuring the zone of inhibition around each well (Excluding the diameter of the well) for the extract. Triplicate tests were conducted against each organism and diameter of zone of inhibition (mm) as expressed as Mean and Standard deviation.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the ethyl acetate and ethanol extracts of *Tectona grandis* L. leaf for the bacterial isolates were evaluated according to the method of Ochei and Kolhatkar (2008), using microtubes dilution method described by National Committee for Clinical Laboratory standards (NCCLS, 2000).

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined by first selecting the tubes that showed no growth during the MIC determination. A loopful from each of the tube was sub-cultured on the sterile nutrient agar and incubated for 24 h at 37°C. The bactericidal effect was demonstrated when no growth occurred on the medium.

Quantitative Phytochemical Screening
Phytochemical screening was carried out on the plant extracts to ascertain the presence of secondary metabolites. Presence of saponin, tannin, alkaloid and flavonoid were determined using the procedure as described by Sofowora (2008).

Determination of the Mineral Composition

The mineral contents of the samples were determined by the procedure of AOAC (2000). Calcium, potassium, magnesium, phosphorus, iron and other elements were measured with Atomic Absorption Spectrophotometer (Thermo scientific S Series Model GE 712354) after digesting with perchloric-nitric acid mixture (AOAC, 2000). Prior to digestion, 5 ml of the samples were measured into a 125 mL Erlenmeyer flask with the addition of perchloric acid (4 mL), concentrated HNO₃ (25 mL) and concentrated sulphuric acid (2 mL) under a fume hood. The contents were mixed and heated gently in a digester at low to medium heat on a hot plate under perchloric acid fume hood and heating was continued until dense white fume appeared. Heating was continued strongly for half a minute and then allowed to cool followed by the addition of 50 mL distilled water. The solution was allowed to cool and filtered completely with a wash bottle into a Pyrex volumetric flask and then made up with distilled water. The solution was then read on Atomic Absorption Spectrophotometer.

Result and Discussion

Quantitative Phytochemical study

In the study, tannins, saponins, alkaloids, flavonoids, glycoside and anthraquinone were observed in the quantitative analysis of the phytochemical study. Flavonoids were most abundant followed by alkaloids and tannins respectively (Tables 1).

Table 1: Phytochemical Analysis of Ethyl acetate and Ethanol Extracts of *T. grandis* leaf (%).

Parameters	Ethyl Acetate Extract	Ethanol Extract
Tannins	3.72	2.16
Saponins	1.83	1.45
Alkaloids	4.16	3.72
Flavonoids	5.89	6.21
Glycoside	0.36	0.28
Anthraquinone	0.44	0.52

Mineral compositions

The mineral compositions of the plant sample are presented in Table 2. Sodium (Na), potassium (K) and calcium (Ca) and iron (Fe) were presented in appreciable quantities in both solvents. But Sodium (Na), Potassium (K), and Calcium (Ca) were highly present in ethyl acetate extract than in the ethanol

extract excluding Iron (Fe), which is more present in the ethanol extract than the ethyl acetate. Moreso, the heavy metals compositions of the plant sample were presented in Table 3. Lead (Pb), Cadmium (Cd) and Arsenic (As) were presented in significant quantities in both extracts of *T. grandis* leaf. However, high amount of the heavy metal's composition was observed in the ethanol extract.

Table 2: Mineral compositions of Ethyl acetate and Ethanol Extracts of *T. grandis* leaf (mg/L).

Parameters	Ethyl Acetate Extract	Ethanol Extract
Sodium (Na)	4.021	3.218
Potassium (K)	2116.083	1761.388
Calcium (Ca)	67.891	63.211
Iron (Fe)	192.456	206.363

Table 3: Heavy metals compositions of Ethyl acetate and Ethanol Extracts of *T. grandis* leaf.

Parameters	Ethyl Acetate Extract	Ethanol Extract
Lead (Pb)	0.068	0.144
Cadmium (Ca)	0.172	0.245
Arsenic (As)	0.865	1.022

Note: All results are represented as mg/L except Arsenic (As) which is represented in $\mu\text{g/L}$.

Antibacterial Activity

Table 4 reveals the antibacterial of the test organisms in ethyl acetate and ethanol extracts of *Tectona grandis* and the control antibiotic. All the test

microorganisms were susceptible to the extracts. Ethyl acetate had the highest antibacterial activity against *Staphylococcus aureus* (70.67 ± 2.082 mm), while the least active was ethanol extract on *Klebsiella pneumonia* (18.00 ± 2.646 mm)

Table 4: Antibacterial Activity of *Tectona grandis* Extracts on some Pathogens.

Organisms	Ethyl Acetate Extract	Ethanol Extract	Antibiotics (Control)
<i>E. coli</i>	67.33 ± 3.055	24.00 ± 2.000	60.00 ± 8.660
<i>Kleb. pneumoniae</i>	18.00 ± 2.646	15.33 ± 1.155	51.00 ± 3.606
<i>Staph. aureus</i>	70.67 ± 2.082	25.00 ± 5.000	61.33 ± 2.082

The Mean with difference along the same row is significant at ($p < 0.05$) Tukey Duncan's Multiple range test. Values are mean of three replicates \pm Standard Deviation.

Minimum Inhibitory Concentration (MIC)

MIC activity of *Tectona grandis* on the microorganisms is presented in Table 5. The same mean zone diameter of 7.29 ± 4.774 mm was observed for all the organisms with both extracts.

However, a mean zone of inhibition of 8.33 ± 3.608 mm was obtained for *E. coli* and *Staphylococcus aureus*, whereas the least mean zone of inhibition of 5.21 ± 1.804 was recorded for *Klebsiella pneumoniae* with the control antibiotics.

Table 5: Minimum Inhibitory Concentration Activity of *Tectona grandis* extracts on some Pathogens.

Organisms	Ethyl Acetate Extract	Ethanol Extract	Antibiotics (Control)
<i>E. coli</i>	7.29 ± 4.774	7.29 ± 4.774	8.33 ± 3.608
<i>Kleb. pneumoniae</i>	7.29 ± 4.774	7.29 ± 4.774	5.21 ± 1.804
<i>Staph. aureus</i>	7.29 ± 4.774	7.29 ± 4.774	8.33 ± 3.608

The Mean with difference along the same row is significant at ($p < 0.05$) Tukey Duncan's Multiple range test. Values are mean of three replicates \pm Standard Deviation.

Minimum Bactericidal Concentration (MBC)

Table 6 presents the highest mean zone diameter of 100 ± 0.000 mm obtained for *E. coli* with ethyl acetate

and *Staphylococcus aureus* with ethanol extract. The control antibiotic had the least mean zone of diameter for all the microorganisms.

Table 6: Minimum Bactericidal Concentration Activity of *Tectona grandis* Extracts on some Pathogens.

Organisms	Ethyl Acetate Extract	Ethanol Extract	Antibiotics (Control)
<i>E. coli</i>	100.00 ± 0.000	83.33 ± 28.868	6.25 ± 0.000
<i>Kleb. pneumoniae</i>	66.67 ± 28.868	66.67 ± 28.868	8.33 ± 3.608
<i>Staph. aureus</i>	50.00 ± 0.000	100.00 ± 0.000	10.42 ± 3.608

The Mean with difference along the same row is significant at ($p < 0.05$) Tukey Duncan's Multiple range test. Values are mean of three replicates \pm Standard Deviation.

The resistance to antibiotics is becoming an increasingly important health and economic menace. However, different antimicrobial substances are elucidated by higher tropical plants (Ogunmefun *et al.*, 2017). The antimicrobial activities of these plants are due to the presence of secondary metabolites present mainly in the leaves, as they are the site for the synthesis of bioactive compounds, hence the presence of high concentrations of active compounds and antibacterial activities in the leaves (Moses *et al.*, 2019). These bioactive compounds, phytochemicals, are known for their interference with the proteins and enzymes of the microbial cell membrane, destroying its structure, leading to the inhibition of various cell functions and the eventual death of the microorganism (Mostafa *et al.*, 2018; Muhammed *et al.*, 2021).

From the results in Tables 4-6, all the microorganisms exhibited varying degrees of susceptibility to both extracts of *Tectona grandis*. The greater mean zone diameter indicated greater antimicrobial effect of the leaf extracts on the microorganisms. The variation could be attributed to the differences in the cell wall composition of the microorganisms. Furthermore, the genetic composition of plasmids which are easily distributed among the microbial strains might account

for these differences (Karaman *et al.*, 2003; Bitchagno *et al.*, 2015). It is surprising to note that the zones of inhibition for the MIC (Table 5) was the same for both extracts of *Tectona grandis* on all the microorganisms, whereas the mean zone diameter of the MBC (Table 6) of both extracts of *Tectona grandis* for the test microorganisms significantly exceeded that of the standard antibiotic (ciprofloxacin). This result can be considered hopeful in view of the need to develop of novel Phyto drugs for combating bacterial infections of public health importance.

Phytochemicals have been reported in several studies to be responsible for the antibacterial potentials of medicinal plants (Preethi *et al.*, 2010; Manimegalai and Rakkimuthu, 2012; Gaikwad and Banerjee, 2013; Jaiswal *et al.*, 2014). In this study, a wide range of phytochemicals such as tannins, saponin, alkaloid, flavonoid, glycoside and anthraquinone were observed. Flavonoids were most abundant followed by alkaloids and tannins respectively (Tables 1). Flavonoids have been recognized as having a protective effect in plants against microbial invasion by plant pathogens. Flavonoids have been shown to possess important biological activities, including antifungal and

antibacterial activities (Kamath and Shabaraya, 2017). However, the ethyl acetate extract had the highest yield of bioactive compounds while the least yield of phytochemical was observed in the ethanol extract.

Sodium (Na), potassium (K) and calcium (Ca) and iron (Fe) were presented in noticeable quantities in both solvents. But Na, K, and Ca were highly present in ethyl acetate extract than in the ethanol extract excluding Fe, which is more present in the ethanol extract. Minerals are essential for proper tissue functioning and a daily requirement for human nutrition (Iniaghe *et al.*, 2009). Minerals acts as a component and activator of many plant enzymes (Paul *et al.*, 2013). Iron is essential in immune functioning, cognitive development, temperature regulation, energy metabolism in their lower concentrations, and is required for the synthesis of haemoglobin and myoglobin while its deficiency causes anaemia (Ikem and Egiebor, 2005; Geissler and Singh, 2011). Calcium is needed for bones and teeth formation and maintenance, blood clotting and muscle contraction (Wardlaw *et al.*, 2004). Sodium is a key factor in retaining body fluid. It helps to make it easier for the small intestine to absorb nutrients like glucose and amino acids. Elevated levels of Na are linked with hypertension and high blood pressure. Potassium is required during glycogenesis, acid-base balance, regulation of osmotic pressure, nerve impulse conduction, and muscle contraction (Njuguna *et al.*, 2013; Paul *et al.*, 2013). Heavy metals are metallic chemical element with a relatively high density that are toxic, poisonous and harmful to human well-being at low concentrations (Sobha *et al.*, 2007). Lead (Pb), cadmium (Cd), and arsenic (As) are typical examples of these heavy metals (Tadesse *et al.*, 2018). In this study, Lead (Pb), cadmium (Cd) and arsenic (As) were presented in significant quantities in both extracts of *Tectona grandis* leaves. However, high amount of the heavy metal's composition was observed in the ethanol extract. As, Cd, and Pb are the most common heavy metals potentially hazardous to human well-being (Lambert *et al.*, 2000). However, cadmium and lead have more significant side effects on human well-being (Hashemi *et al.*, 2017). The trivial amounts of heavy metals exhibited, suggests that there are not toxic to the body.

Conclusion

This study revealed that *Tectona grandis* (teak) leaves extracts studied has antibacterial activity on pathogens causing upper respiratory tracts infections (URTIs), in

particular *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The plants also contained various phytochemical, minerals and in sufficient quantities of heavy metals thereby validating the use of this plants for therapeutic tenacities.

Acknowledgements

We are grateful to the Department of Science Laboratory Technology, D. S. Adegbenro ICT Polytechnic, Itori, Ewekoro, Ogun State for the infrastructural facilities and access to scientific instrumentation. We acknowledge Mr. Bankole Ayomipo Oluwakunmi for assisting with the phytochemical analysis of the plant materials, thank you so much.

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