A study on the biocidal potentials of stem bark extract of *Leucaena leucocephala* (Lam.) on some selected bacterial strains

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Abstract

Leucaena leucocephala is a fast-growing leguminous plant this is used in the treatment of intestinal diseases and as birth control. This work was geared toward investigating the phytochemical attributes and the antimicrobial actions of methanol and distilled water crude extracts from the stem bark of *Leucaena leucocephala* in the ratio of 3:2 on nineteen bacterial species. The antimicrobial activity of the plant extract was done with the use of the agar diffusion technique in line with the National Committee for Clinical Laboratory Standards. The extract was located to be energetic against sixteen bacterial traces at a concentration of 35 mg/mL except for *Bacillus anthracis, Clostridium sporogenes,* and *Pseudomonas fluorescens*. The zones of inhibition exhibited by means of the extract towards the organisms ranged from 10.00 mm to 20.00 mm. The MIC exhibited by the extract ranged from 2.19 mg/ml to 8.75mg/ml while the MBC ranged from 4.38 mg/ml to 17.5 mg/ml. The effects of the extract on bacterial species were compared with the use of selected antibiotics such as streptomycin, and ampicillin. The phytochemical parts of the extract include tannins, glycosides, alkaloids, phenols, and terpenoids. Results from this research confirmed that the stem bark extract of *Leucaena leucocephala* exhibited antimicrobial activity against the selected bacteria and support the usefulness of this plant in folklore treatments.

***Keywords*:** *Leucaena leucocephala,* Biocidal, Phytochemical, Antimicrobial activities

1. Introduction

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contain what we would currently characterize as antimicrobial principles, was well accepted. Man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies (Enerijiofi *et al*., 2021; Heinrich *et al*., 2004).

Leucaena leucocephala belongs to the family Fabaceae and is one of the fastest-growing leguminous trees in drought-prone and semi-arid areas (Sethi *et al*., 1995). It is commonly referred to as Lead Tree or River Tamarind. It is planted as a shade tree for coffee, cacao, and other cash crops; for soil fertility improvement; erosion control; site preparation in reforestation and used for a variety of other purposes including timber and fuel wood.

A toxic, non-protein amino acid in *L. Leucaena,* causes alopecia, growth retardation, cataract, goiter, decreased fertility, and mortality in non-ruminants. The mechanism of this toxicity is complicated. An acylated flavonol glycoside along with seven flavonoids was isolated for the first time from the dried leaves of *L. leucocephala* (Mohammed *et al*., 2015). This plant also possesses antioxidant property and has also revealed a reducing agent, metal chelator, hydrogen donating ability. Sulphated Polysaccharide from seeds of *Leucaena leucocephala* was reported to have cancer chemopreventive and anti-proliferation activity against human hepatocarcinoma (Gamal-Eldeen *et al*., 2007).

Medicinal plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases. The studies of medicinal plants used in folklore remedies have attracted the attention of many scientists in finding solutions to the problems of multiple resistances to the existing synthetic antibiotics. Most of the synthetic antibiotics now available in the market have major setback due to the multiple resistance developed by pathogenic micro-organisms against these drugs. Thus, there is a need to search for new and more potent antimicrobial compounds of natural origin to combat the activities of these pathogens (Akinpelu *et al*., 2008)

Presently, there are global problems of multiple antibiotics resistance as well as the emergence of new and resurrection of previously eradicated diseases. Reports on ethno botanical records indicate a general consensus on the use of antimicrobial active medicinal plants to provide cheaper drugs. There is need to search for new and more potent antimicrobial compounds of natural origin to complement the existing synthetic antimicrobial drugs that are gradually becoming less potent against pathogenic microorganisms (Akinpelu *et al*., 2008; Enerijiofi *et al*., 2019).

The World Health Organization (WHO) estimates that approximately 80% of the world's inhabitants rely on traditional or herbal medicines for their primary health care and plants have long formed the basis of sophisticated traditional medicine systems and purportedly provide excellent leads for new drug developments (Akinjogunla *et al*., 2009). Herbal medicine is the oldest form of healthcare known to mankind and over 50% of all modern clinical drugs are of natural products origin and natural products play important roles in drug development in the pharmaceutical industry (Preethi *et al*., 2010).

The rediscovery of the connection between plants and health is responsible for the launching of a new generation of multicomponent botanical drugs, dietary supplements and plant produced recombinant proteins (Akinjogunla *et al.*, 2011). However, the increasing problems of multidrug resistant (MDR) bacteria is of great concern to both the clinicians and pharmaceutical industries and this has made it significant to search for newer drugs that are highly effective, affordable, acceptable and available.

The inappropriate use of antibiotics is a major driver of antimicrobial resistance (AMR) and is a considerable public health concern across many continents and countries including sub-Saharan Africa (Ayukekbong *et al*., 2017). As a result, the Global Action Plan on Antimicrobial Resistance was adopted in May 2015 by the World Health Assembly and endorsed by Heads of States of many countries, including Nigeria, at the United Nations General Assembly in New York in September 2016 to try and reduce AMR rates. This is because AMR increases health care costs and the economic burden on individuals, families, healthcare systems and society since antimicrobial resistant infections are more difficult to treat with a tendency for longer hospital stays and increased mortality (Hofer, 2019).

In recent years, the role of AMR caused by methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin-Resistance Enterococci (VRE) and extended-spectrum beta-lactamases (ESBL) in 3 increasing patients' morbidity and mortality is a matter of public health concern (Bassetti *et al*., 2019). Across countries, inappropriate prescribing and dispensing of antibiotics across all healthcare sectors continues to be reported, particularly in low-middle income countries (Ding *et al*., 2019). The link between quantity of antibiotics consumed and it resistance has been reported in studies conducted in several countries (Llor and Bjerrum, 2014).

Plant extracts and essential oils are reported to be useful as natural antimicrobial agents. Many plant-derived compounds are reported to exhibit promising results against methicillin-resistant bacteria and can reverse antibiotic resistance. These antimicrobials, if administered at a recommended level, can decrease antibiotic resistance because plant extracts can be used to reverse antibiotic resistance (Sallam *et al*., 2021).

This study was designed with the intent of evaluating the antibacterial potency of the crude extract of the stem bark of *Leucaena leucocephala* with a focus of on evaluating the antimicrobial potency of the plant while also noting the minimum inhibitory concentration and minimum bactericidal concentration of susceptible bacteria isolates to provide more ways of combating the problems possed by the usage of antibiotics. The compounds present in the stem bark of *Leucaena leucocephala* extract responsible for its antimicrobial activity were also identified.

2. Materials and methods

2.1 Sample Collection

Plant materials

The stem bark of *Leucaena leucocephala* used for this study was collected from Botanical Garden at Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The plant sample was identified at the Herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Figure 1: *Leucaena leucocephala* Plant

Microorganisms

The following bacterial isolates used for this research were obtained from the microbial collection of Prof. D. A Akinpelu, Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Bacillus anthracis (LIO), *Bacillus cereus* (NCIB 6349), *Bacillus polymyxa* (LIO), *Bacillus steareothermophilus* (NCIB 8222), *Bacillus subtilis* (NCIB 3610), *Clostridium sporogenes* (NCIB 532), *Corynebacterium pyogenes* (LIO), *Enterococcus faecalis* (NCIB 775). *Escherichia coli* (NCIB 86), *Klebsiella pneumoniae* (CISP), *Klebsiella pneumoniae* (NCIB 418), *Micrococcus luteus* (NCIB 196), *Proteus vulgaris* (LIO), *Pseudomonas aeruginosa* (CIW), *Pseudomonas aeruginosa* (NCIB 950), *Pseudomonas fluorescens* (NCIB 3756), *Shigella* sp. (LIO), *Staphylococcus aureus* (NCIB 8588), *Streptococcus pneumoniae* (CIB)

Key:
NCIB: National Collection of Industrial Bacteria, LIO: Locally Isolated Organism, CIB: Clinical Isolate from Blood, CIW: Clinical Isolate from Wound, CISP: Clinical Isolate from Sputum.

2.2 Drying and extraction of plant substances

Leucaena leucocephala stem bark was air-dried until a constant weight of the sample was obtained. The dried sample was ground into fine powder. Exactly 150 g of the powdered sample was soaked in methanol and sterile distilled water in a ratio of 3:2 (v/v) for four days on the laboratory bench with regular agitation. The supernatant collected was then filtered with glass wool into a sterile clean flask. The filtrate was concentrated in a vacuo using a rotary evaporator to remove the organic solvent. The aqueous part was then lyophilized giving a brownish powdery crude extract Equal volume each of both ethanol and aqueous extract was added to 5ml of ethanol and distilled water respectively to give a concentration of 35 mg/ml (Shanthi *et al*., 2021).

2.3 Preparation of selected bacterial strains for the experiment

The bacterial strains used for the experiment had been re-activated in nutrient broth and incubated at 37ºC for 18 h before use. The bacterial strains were standardized before use using the McFarland standard.

2.4 Antimicrobial sensitivity testing of crude stem bark extract of *L. leucocephala* against selected bacterial strains.

The sensitivity testing of the extract was carried out using the agar-well diffusion method as described by Rusell and Furr (1997); Akinpelu *et al*., (2015). The bacterial strains were grown in nutrient broth for 18 h and standardized using McFarland Standard (10^{-6} CFU/mL or 0.5 McFarland standard) before use. The inoculum was then streaked onto an already sterilized Mueller-Hinton agar plate. Wells were bored on the agar medium aseptically using a sterile 6 mm cork borer. The wells were filled with the solutions of the extracts of predetermined concentration. The final concentrations of the crude extract and the fractions used were 35mg/ml. On the other hand, ampicillin and streptomycin were used as positive controls at a concentration of 1 mg/mL. The plates were left on the laboratory bench for 1 h to allow proper in-flow of the solution into the medium before incubating them at 37°C for 24 h. The plates were not stock-piled to allow even distribution of temperature around the plates to avoid false results. The plates were later observed for zones of inhibition which is an indication of the susceptibility of the test organisms to the extracts. Ampicillin and streptomycin were used as positive controls. Proper care was taken not to allow the solution of the extract to spill on the surface of the agar plates

2.5 Determination of minimal inhibitory concentrations (MICs) of stem bark extract of *L. leucocephala* on test organisms.

The MICs of the extract was determined using two-fold dilution as described by Akinpelu and Kolawole (2004). Two-fold dilution of the extract was prepared and 2 mL of different concentrations of the solution was added to 18 mL of pre-sterilized molten nutrient agar to give final concentration range of 0.274 mg/ml to 17.5 mg/mL. The medium was then poured into sterile Petri dishes and allowed to set. The surfaces of the media were allowed to dry before streaking with 18 h standardized bacterial cultures. The plates were later incubated at 37ºC for up to 72 h after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the bacterial strains.

2.6 Determination of minimal bactericidal concentrations (MBCs) of the stem bark extract of *L. leucocephala* on test organisms.

Minimum bactericidal concentration of the extracts was determined using Olundare *et al.*, 1992 with little modification. Samples for the MBC were taken from the line of streak on MIC plates without visible growth and then streaked on a freshly prepared nutrient agar medium plates and incubated at 37ºC for 48 h. The plates were later examined for the presence or absence of growth. The MBC was taken as the lowest concentration of the extract that did not allow any bacterial growth on the surface of the agar plates.

2.7 Phytochemical Analysis

A small portion of the dry extract was subjected to phytochemical test using Trease and Evans (2002); and Enerijiofi and Isola (2019) techniques to test for alkaloids, tannins, flavonoids, saponins, terpenoids, and cardiac glycoside.

3. Results

The crude extract obtained from 200 g of powdered stem bark of *L.leucocephala* was dark brown in colour. The yield was 18.5 g which was 12.3% of the powdered sample. Table 1 shows the results exhibited by the stem bark extract of *L. leucocephala* against the bacterial strains. The extract was found to be active against sixteen out of the nineteen organisms tested at a concentration of 35 mg/mL. The zones of inhibition exhibited by the extract against the test organisms ranged between 10.00 mm to 20.00 mm. The minimum inhibitory concentrations and minimum bacterial concentrations of the stem bark extract of *L. leucocephala* against susceptible bacterial strains were determined (Table 2).

The MIC exhibited by the extract ranged between 2.19 mg/mL to 8.75 mg/mL, while, minimum bactericidal concentration ranged between 4.38 mg/mL to 17.5 mg/mL. The result of the phytochemical analysis based on the chemical examination of the stem bark extract of *L. leucocephala* is as indicated in Table 3.

Table 1: Antimicrobial sensitivity testing exhibited by *L. leucocephala* stem bark extract against selected bacterial strains.

Microorganisms	**Zones of inhibition (mm)***			
	Crude extract (35 mg/mL)	**Strept (1 mg/mL)**	**Ampicillin (1 mg/mL)**	**M/W (1:1)**
Bacillus anthracis (LIO)	0.00	17.00	0.00	0
Bacillus cereus (NCIB 6349)	11.00	20.00	0.00	0
Bacillus polymxya (LIO)	11.00	12.00	0.00	0
Bacillus stearothermophilus (NCIB 8222)	10.00	20.00	0.00	0
Bacillus subtilis (NCIB 3610)	10.00	20.00	15.00	0
Clostridium sporogenes (NCIB 532)	0.00	12.00	0.00	0
Corynebacterium pyogenes (LIO)	11.00	12.00	0.00	0
Enterococcus faecalis (NCIB 775)	12.00	0.00	0.00	0
Escherichia coli (NCIB 86)	11.00	0.00	0.00	0
Klebsiella pneumoniae (CISP)	10.00	0.00	15.00	0
Klebsiella pneumoniae (NCIB 418)	14.00	20.00	0.00	0
Micrococcus luteus (NCIB 196)	15.00	21.00	0.00	0
Proteus vulgaris (LIO)	10.00	18.00	0.00	0
Pseudomonas aeruginosa (CIW)	11.00	20.00	15.00	0
Pseudomonas aeruginosa (NCIB 950)	20.00	24.00	0.00	0
Pseudomonas fluorescens (NCIB 3756)	0.00	19.00	0.00	0
Shigella sp, (LIO)	11.00	20.00	14.00	0
Staphylococcus aureus (NCIB 8588)	11.00	25.00	12.00	0
Streptococcus pneumoniae (CIB)	12.00	0.00	0.00	0

Keys:M/W: Methanol Water, 0 = Resistant, NCIB = National Collection of Industrial Bacteria, CISP = Clinical Isolate from Sputum LIO = Locally Isolated Organism, CIB = Clinical Isolate from Blood, CIW = Clinical Isolate from Wound.

mm* = mean of three replicate readings.

Table 2: Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) exhibited by *L. leucocephala* extract against susceptible bacterial strains

Microorganisms	MIC (mg/mL)	MBC (mg/mL)
Bacillus cereus (NCIB 634)	8.75	17.5
Bacillus polymyxa (LI0)	4.38	8.75
Bacillus stearothermophilus (NCIB 8222)	8.75	17.5
Bacillus subtilis (NCIB 3610)	8.75	17.5
Corynebacterium pyogenes (LIO)	4.38	8.75
Enterococcus faecalis (NCIB 775)	4.38	8.75
Escherichia coli (NCIB 86)	4.38	8.75
Klebsiella pneumoniae (CISP)	8.75	17.5
Klebsiella pneumoniae (NCIB 418)	4.38	8.75
Micrococcus luteus (NCIB 196)	4.38	8.75
Proteus vulgaris (LIO)	8.75	17.5
Pseudomonas aeruginosa (CIW)	8.75	17.5
Pseudomonas aeruginosa (NCIB 950)	2.19	4.38
Shigella sp, (LIO)	8.75	17.5
Staphylococcus aureus (NCIB 8588)	8.75	7.50
Streptococcus pneumoniae (CIB)	4.38	8.75

Table 3: Phytochemical compounds present in the stem bark extract of *L. leucocephala*

Phytochemical Compounds	Results
Glycosides	+ve
Alkaloids	+ve
Tannins	+ve
Saponins	-ve
Flavonoids	-ve
Terpenoids	+ve
Sterols	-ve
Phlobatannins	-ve
Resins	-ve
Carbohydrates	-ve
Phenols	+ve

4. Discussion

The antimicrobial activity of the stem bark extract of *L. leucocephala* was determined against nineteen bacteria strains comprising both Gram positive and Gram negative bacteria. The extract was found to

be active against sixteen of the nineteen bacterial strains at a concentration of 35 mg/ml. The extract exhibited activity against both Gram positive and Gram negative bacteria and thus exhibited a broad spectrum activity. Plant like L. *leucocephala* exhibiting such action could serve as a pointer towards development of antimicrobials to combat the action of pathogens causing infections in humans (Erhabor *et al.*, 2017).

The extract *from L. leucocephala* exhibited significant activity against Gram-negative bacteria especially *Escherichia coli* and *Salmonella typhimurium* (Mohammed *et al*., 2015). Aderibigbe *et al*. (2011) showed that *L. leucocephala* seed oil extract inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis, Pseudomonas aeruginosa*, and *Escherichia coli*. The extracts of *L. leucocephala* possess significant antimicrobial and antimutagenic potentials. These activities might be attributed to the presence of effective phytoantimutagens and phytochemicals. These bioactive compounds belong to a variety of different chemical groups such as phenolics, pigments, allylsulfides, glucosinolates, tannins, anthocyanins, flavonoids, phytosterols, protease inhibitors, and phytoestrogens (Chaurasia *et al*., 2015).

Various parts of *L. leucocephala* have been reported to have medicinal properties ranging from control of stomach diseases to contraception and abortion and the seed gum has been reported to be useful as a binder in tablet formulation (Verma and Balkishem, 2007). *Leucaena leucocephala* leaves were found to have inhibitory activity on *Micrococcus luteus*, methicillin-sensitive *Staphylococcus aureus* (Chew *et al*., 2011).

The result of the phytochemical analysis of the plant extract revealed the presence of glycosides, terpenoids, alkaloids, tannins, and phenols. Phytochemicals are natural and non-nutritive bioactive compounds produced by plants that act as protective agents against external stress and pathogenic attack. Phenolic is one of the major groups of phytochemicals that can be found ubiquitously in certain plants. Phenolic compounds are potent antioxidants and free radical scavenger which can act as hydrogen donors, reducing agents, metal chelators and singlet oxygen quenchers (Chew *et al.*, 2009).

Tannins another phytochemical in *L. leucocephala* extract acts by iron deprivation, hydrogen binding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991). Herbs that contain tannins as a component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery thus exhibiting antimicrobial activity (Dharmananda, 2003). Thus, the presence of these phytochemicals, phenolic compounds and tannins contributed to the bioactive action of *L. leucocephala* stem bark extract.

Alkaloids are organic compounds that contain nitrogen and are physiologically active with sedatives and analgesic properties. Glycosides are compounds containing carbohydrate and non-carbohydrate residue (moiety) in the same molecule. They all contain steroid as aglycone component in combination with a sugar molecule, Cardiac glycosides are used in the treatment of congestive heart failure and cardiac arrhythmia (Awe *et al*., 2013). Alkaloids are the largest group of secondary chemical constituents and most efficient plant substances used therapeutically because of their well-documented antimicrobial and antihelmintic activity. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agent because of their analgesic and antiplasmodic activity (Okwu *et al*., 2004). They also possess anti-inflammatory properties (Staerk *et al*., 2002). These findings contributed to the reasons why *L. leucocephala* is used as folklore remedies for the treatment.

The presence of these phytochemicals in the stem bark extract of *Leucaena leucocephala* is most likely to be responsible for its antibacterial properties. Presence of tannins in the plant extract is known to aid wound healings as documented in some literatures. The MIC and MBC of *L. leucocephala* extract were also determined. The extract exhibited lower MIC and MBC values. This is an indication of a good antimicrobial activity. Plants with low MIC and MBC are good indicator of potent antimicrobial activities (Akinpelu *et al*., 2015).

5. Conclusion

The results obtained from this study against some selected pathogens associated with human infections serves as a pointer towards the development of antimicrobials and antioxidant from stem bark of *L. leucocephala*. Such drugs of natural origin will go a long way in healthcare delivery.

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Conflict of interest

Authors declare that they have no conflicts of interest.

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