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Characterization, Bioactive Profiling and Evaluation of the Pharmacological Activities of Ethanol Extract of *Lannea welwitschii* (Hiern) Engl.

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Lannea welwitschii* is a medicinal plant used in traditional medicine to treat various infectious diseases due to its plethora of bioactive compounds that possess various pharmacological activities. This study aimed to characterize and determine the phytochemical profile and the bioactive compounds responsible for their pharmacological activities.

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Methods: Phytochemical screening and Gas-Chromatography Mass Spectrometry (GC-MS) analysis were carried out on the ethanolic extract of *L. welwitschii* to determine the volatile compounds present. Characterization of the crude extract was carried out using Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-ray diffraction (XRD) and Energy Dispersive X-ray spectroscopy (EDX). The antioxidant potential of the extract was evaluated using methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant Potential (FRAP). The antibacterial activities were determined using the macro-broth dilution technique.
Results: Secondary metabolites such as saponins, tannins, alkaloids, reducing sugars, and cardiac glycoside were identified. The GC-MS analysis showed the presence of 48 bioactive compounds, out of which 12 had peak area percentages ≥ 1%. Some of the bioactive compounds identified include 2-butoxy-ethanol, Dodecanoic acid, Stigmasterol, Isopropenyl, and n-hexadecanoic acid, which have been reported with different biological activities. FTIR analysis revealed the presence of various functional groups which showed major compounds in the plant extract. The morphological features showed the spherical shape of the plant extract with several aggregates, while the EDX analysis identified elements such as silicon, oxygen, and silver. The extract demonstrated inhibitory potential against some of the bacterial isolates. However, *Bacillus pumilus, Klebsiella quasipneumoniae*, *Klebsiella aerogenes,* and *Staphylococcus aureus* are more susceptible with MIC of 0.313 and 0.625 mg/ml respectively.
Conclusion: The presence of secondary metabolites and bioactive compounds revealed by the characterization and phytochemical analysis provided enough evidence for the usage of the plant in the treatment of infectious diseases and thus might be considered a therapeutic agent.

Keywords: Lannea welwitschii; phytochemical; GC-MS analysis; pharmacological activities.

1. INTRODUCTION

"The use of plants as medicines by humans could be dated back to the middle Paleolithic age, about 60,000 years ago, according to fossil records" (Fabricant & Farnsworth, 2001). Plants' parts including leaves, stems, roots, and berries are sources of many potent and powerful medications that have been used in healthcare for therapeutic or medicinal purposes (Iwu, 1993). "The World Health Organisation estimated that 80% of the world's population, especially in underdeveloped nations, still uses medicinal plants as their primary source of healthcare. Many plants used in folkloric medicines, in the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations have shown powerful antimicrobial and antioxidant activities and possess bioactive characteristics" (Ozioma & Chinwe, 2019; Adeoye-Isijola et al., 2022; Animashaun et al., 2023; Fagbemi et al., 2023). They have been found effective in treating a variety of human illnesses including cancer, cardiovascular disease, and inflammatory diseases (Shedoeva et al., 2019) because their antioxidant activities have been able to lessen oxidative stresses in cells and mop up reactive oxidative species.

"Although medicinal plants possess medicinal properties, they are known to be rich in novel compounds that can be used for drug synthesis. The novel compounds producing physiological effects on living organisms are stored as active chemical compounds" (Phillipson, 2001; De Boer & Cotingting, 2014; Subramanian & Saratha, 2010; Nithyadevi & Sivakumar, 2015; Roger et al., 2015). "Among the bioactive compounds, triterpenoids provide anti-inflammatory activity and tannins possess astringent, anti-inflammatory, and antimicrobial properties" (Kennedy & Wightman, 2011). "While saponins often have medicinal features such as blood cleansers, expectorants, and antibiotics properties, alkaloids have significant effects on the central nervous system (CNS) and glycosides are known for their ability to increase the forces of systolic concentration" (Olaitan et al., 2013).

Over the last few decades, the use of herbal drugs has been emphasized due to their availability, therapeutic potential, minimal side effects and reduced cost of treating infections as against microbial infections being frequently treated with synthetic medications (Salem et al., 2013; Kabuto et al., 2004; Rajamanikandan et al., 2022; Nisha et al., 2010). But sadly, due to the indiscriminate use of these anti-infective agents, microorganisms have become resistant to many antibiotics. Pharmacologically, these antibiotics can also impair immunological

function and occasionally trigger allergic responses. On the contrary, Golshani et al. (2015) indicated that the therapeutic use of plants is safer for both the environment and human health. “Many compounds isolated from these plants have been used as drugs either in their natural form or in semi-synthetic form *Lannea welwitschii* (Hiern) Engl. which belongs to the family Anacardiaceae, is a medium-sized woody tree that is commonly found in tropical regions of the world, especially in deciduous and secondary forests” (Ebanyenle et al., 2005). “*L. welwitschii*, commonly called Ekika or Ewinwan in Nigeria, is used in most traditional medicines in West Africa to manage diverse forms of infections and chronic diseases” (Olatokunboh et al., 2009; Ebabhi et al., 2014; Waly et al., 2015; Gao et al., 2018). “In the past few decades, it has become the focal point of research for newer and more potent therapeutic agents in some parts of the world, especially the tropical regions, due to its numerous, traditional uses for medicinal purposes (Elbadrawy & Sello, 2016; Ambavade et al., 2014; Nogueira et al., 2019; AL-Jawad et al., 2017; Xiao et al., 2020). While the plant is widely used to treat diverse forms of infections and chronic diseases, it has been reported in different studies to possess antimicrobial, antioxidant, anti-inflammatory, analgesic, antidiabetic, antiallergic, antidiarrhoeal, and cytotoxic pharmacological activities” (Amole & Ilori, 2010; Agyare et al., 2013; Pivetta et al., 2018; Shahbazi, 2019; Ghazala et al., 2009; Baroroh et al., 2023; Chua et al., 2021). This plant's pharmacological activities have been attributed to several phytochemicals such as alkaloids, saponin, tannin, and flavonoids in the plant. However, there is a dearth of information on the phytochemical constituents and chemical compounds in its ethanol extract (Awwad et al., 2013; Sivakumar et al., 2011; Zuzarte & Salgueiro, 2015; Amiranashvili et al., 2020). This study, therefore, investigated the antioxidant and antibacterial activities as well as the phytochemical constituents and chemical compounds present in the ethanol extract of *L. welwitschii* using the GC-MS technique.

2. MATERIALS AND METHODS

Plant materials and collection: Fresh stem barks of *L. welwitschii* were obtained from its natural habitat in Ilishan-Remo, Ikenne Local Government area, Ogun state, Nigeria. Plant material was identified and authenticated at the Forestry Research Institute of Nigeria, Ibadan (FRIN) with specimen no FHI 113398 and was deposited at the herbarium.

Preparation of plant samples: The stem bark collected was air-dried at room temperature (28 ℃) and pulverized into a coarse powder using a mechanical grinder before being extracted with ethanol. Twenty gram (20 g) of the powdered stem bark was extracted with 200 mL of ethanol and placed on a shaker for 24 h. The extract was filtered with Whatman No. 1 filter paper after which the extracting solvent was evaporated from the mixture with rotary evaporator before being further dried in the oven in a sterile pre-weighed beaker at 40 ℃ to obtain a solvent-free dried extract residue

Phytochemical screening: The ethanol extract of *L. welwitschii* was subjected to both qualitative and quantitative phytochemical analyses to identify different secondary metabolites like alkaloids, tannins, steroids, flavonoids, terpenoids, glycosides, phlobatannins, phenols, saponins, and reducing sugars ((Soladoye & Chukwuma, 2012; Wintola et al., 2021; Saad et al., 2022; Sharma et al., 2020; Kandasamy et al., 2014).

2.1 Quantitative Phytochemical Analysis

Determination of saponin: The quantitative determination of saponin was performed using the methodology described by Ejikeme et al. (2014) with modifications. Two grams of pulverized plant materials was weighed into a test tube containing 20 mL of water placed in a water bath, and then filtered. The filtrate (10 mL) was then mixed with 5 mL of the ethanol used as extracting solvent, agitated vigorously and allowed to stand for 15 min. The frothing was mixed with three (3) drops of olive oil, agitated vigorously and observed for the formation of emulsion to indicate the presence of saponin.

Determination of phenol: “Two grammes (2 g) of the pulverized plant sample was defatted for 2 h in 100 mL of ether using a Soxhlet apparatus. 0.50 g of the defatted sample was boiled for 15 min with 50 mL of ether for the extraction of the phenolic components. Ten millilitres (10 mL) of distilled water, 2 mL of 0.1N ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were added to 5 mL of the extract and left to react for 30 min to observe colour change and optical density was measured at 505 nm. Tannic acid (0.20 g) was dissolved in distilled water and diluted to 200 mL mark in preparation for the phenol standard curve. Different concentrations of the standard tannic acid (0.2-1.0 mg/cm^3) were pipetted into five

different test tubes to which 2 cm^3 of NH_3OH, 5 mL of amyl alcohol and 10 mL of water were added. The solution was made up to 100 cm^3 volume and left to react for 30 min for colour development. The optical density was determined at 505 nm with UV/VIS TG 50 spectrophotometer" (Aneta et al., 2013).

Determination of alkaloids: Two hundred millilitres (200 ml) of 10% acetic acid in ethanol was added to 5 g of each plant sample. The mixture was then covered and allowed to stand for 4 h. The extract was then concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added until precipitation was completed immediately after filtration. After 3 h of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 0.1 mL of ammonium hydroxide and then filtered. The residue was then dried and weighed in an oven and the percentage of alkaloid was calculated,

$$\% \text{ Alkaloid} = \frac{\text{Weight of alkaloid X 100}}{\text{Weight of sample}}$$

Determination of flavonoids: Finely ground plant extract (2.50 g) was combined with 100 ml of 80% aqueous methanol and left to stand for 24 h at room temperature. The residue was extracted again using the same amount of ethanol after the supernatant was discarded. The solution was then filtered, and the filtrate was then dried over a water bath. The substance was desiccator-cooled before being weighed to determine its constant weight (Ejikeme et al., 2014).

$$\% \text{ Flavonoid} = \frac{\text{Weight of flavonoid X 100}}{\text{Weight of sample}}$$

Determination of tannins: In 37 mL of distilled water, 50 g of sodium tungstate (Na_2WO_4) was dissolved. Orthophosphoric acid (H_3PO_4) (25 mL) and 10 g of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) were added to the Folin-Denis reagent. It was then chilled, and diluted to 500 mL with distilled water after 2 h of refluxing. One gram (1 g) of each pulverized plant extract was added to 100 mL of distilled water, boiled gently for 1 h, and filtered using Whatman No. 1 filter paper. Five millilitres (5 mL) of Folin-Denis reagent and 10 mL of saturated Na_2CO_3 solution were added into 50 mL of distilled water and 10 mL of diluted extract was carried out after being pipetted into a conical flask and observed for colour development. The solution was allowed to stand for 30 min in a water bath at a temperature of 25 ℃ after thorough agitation. The optical density was determined at 700 nm using a UV/VIS spectrophotometer, and comparisons were made using a typical tannic acid curve. The standard tannic acid solution was pipetted into five different test tubes at various concentrations (0.2-1.0 mg/cm^3). The solution was then mixed with 5 mL of the Folin-Denis reagent and 10 mL of saturated Na_2CO_3, and left to stand for 30 min in a water bath at 25 °C. Tannic acid concentration was plotted against optical density (absorbance).

$$\text{Tannic acid (mg /100 g)} = \frac{C \times \text{extract volume} \times 100}{\text{Aliquot volume} \times \text{weight of sample}}$$

where C is the concentration of tannic acid read off the graph

Identification of chemical compounds using GC-MS: The ethanol extract of *L. welwitschii* was subjected to GC-MS analysis. The GC-MS analysis of the extract was carried out in a (QP 2010 plus SHIMADZU) instrument under computer control at 70eV. One microliter (1 µl) of the ethanol extract was injected into the GC-MS using a micro-syringe and the scanning was done for 24 min. Once the separation of the compound began, migration from the column was entered into a detector programmed to give a signal whenever a compound was detected. Chromatographs was generated in form of a graph from the signal received from the detector while the instrument was running. Compounds that emerged from the gas chromatographic column was heated and separated. The electron ionization (mass spectrometry) detector was identified based on the mass of each compound. The compound identification was performed by using the database. "The spectrum of the unknown components was compared with the spectrum of known components stored in the library. The name, molecular weight and structure of the components of the test materials were ascertained. Compounds in the extract were identified using the National Institute of Science and Technology (NIST) database (NIST/EPA/NIH mass spectral library (2014). The average peak area of the total areas was calculated for comparing relative percentage amount of each component" (Fagbemi et al., 2022; Yoshida & Niki, 2003; Gupta et al., 2021; Nzogong et al., 2018).

FTIR analysis: Fourier transform infrared spectroscopy (FTIR) analysis (Perkin-Elmer) was carried out to identify the likely biomolecules

present in the plant extract using an FTIR spectrophotometer. A few crystals were combined with potassium bromide (KBr) (Merck for spectroscopy) and grounded in an agate mortar to create a homogenous powder from which the suitable pellet was created under a pressure of 7 tons. Using the Pelkin Elmer 3000 MX spectrometer, all spectra were captured between 4000 and 400 cm^{-1}. Each spectra had 32 scans with a resolution of 4 cm^{-1}. Win-IR Pro Version 3.0, a spectroscopic program with a peak sensitivity of 2 cm^{-1}, was used to analyze the IR spectra (Raba et al., 2015).

Scanning Electron Microscopy (SEM) and Energy-dispersive X-ray spectroscopy analyses (EDX): The plant extract's surface morphology was identified using scanning electron microscopy. After 6 h of reaction, the sample was made by centrifuging colloidal solution at 14,000 rpm for 4 min. After being re-dispersed in deionized water and centrifuged once more, the pellet was obtained. After three iterations of the procedure, the material was ultimately cleaned with acetone. A drop of the solution was then applied to the copper grid with carbon coating after the plant extract had been sonicated for 10 min. The sample was dried fully under a lamp the entire time. After obtaining the SEM image, photomicrographs were taken at different magnifications then the extract was passed through an EDX detector to determine the elemental composition. Field emission SEM (FESEM, Carl Zeiss and Supra 40) was used in this investigation to carry out microstructural characterization (Wibawa et al., 2020).

X-ray diffraction Analysis (XRD): The particle size and the nature of extract were determined using X-ray diffraction (XRD) analysis. Powdered samples of the mixture were pelletized, sieved to 0.074 mm and was subjected to an intense X-ray beam at a scanning rate of 2°/min in the 2^{o} to 50° at room temperature with a Cu Kα radiation set at 40 kV and 20 mA. The peaks obtained were analysed according to the intensities and was compared to that of standard data of minerals from the mineral powder diffraction file in International Centre for Diffraction Data (ICDD) which contained and includes the standard data of more than 3000 minerals (Wibawa et al., 2020; Mohiuddin et al., 2022; Rajkumar et al., 2018).

Antioxidants activity: The antioxidant activity of the ethanol extract of *L. welwitschii* was determined using standard methods with modifications (Lu et al., 2008; Panda et al., 2010; Kattamis et al., 2011). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assay, Ferric reducing antioxidant potential assay (FRAP), Nitric oxide scavenging activity, and lipid peroxidation inhibitory assay were antioxidant assays carried out in this study.

DPPH radical scavenging assay: "For DPPH assay, 0.1 mM solution of DPPH in ethanol was prepared, and 1 mL of the solution was added to 1 ml of extract in water at different concentrations (25-100 µg/mL). The mixture was then shaken vigorously and allowed to stand at room temperature for 30 min. Absorbance was measured at 517 nm by using a UV-visible spectrophotometer. The percentage inhibition of free radical in DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of the sample)/absorbance of control] × 100%. All the tests were carried out in triplicates. Though the activity was expressed as 50% inhibitory concentration (IC_{50}), IC_{50} was calculated based on the percentage of DPPH radicals scavenged. The lower the IC_{50} value, the higher the antioxidant activity" (Sonboli et al., 2005).

Ferric reducing power assay: "The spectrophotometric method was used for the measurement of reducing powder. One millilitre of the extract sample was mixed with 2.5 mL of 0.1 M Sodium phosphate buffer (pH 6.6) and 2.5 ml of 1%, w/v Potassium ferrocyanate [$K_3Fe(CN)_6$] in a 250 mL conical flask and then incubated at 50°C for 20 min. Thereafter, 2.5 mL trichloroacetic acid (10%, w/v) was added and the mixture was centrifuged at 5000 rpm for 10 min. The upper layer (5 mL) was mixed with 0.5 mL of fresh $FeCl_3$ (0.1%, w/v), and the absorbance was measured at 700 nm against a blank in a spectrophotometer. Increasing absorbance of the reaction mixture indicated greater reducing power of the sample. Gallic acid was used as the control" (Otang et al., 2012).

FRAP Scavenging effect (%) = [(A0-A1)/A0] x 100

Nitric oxide radical scavenging assay: Ten milligrams per mL (10 mg/mL) of the sample were serially diluted with distilled water to make concentrations from 25-100 µg/mL and the standard ascorbic acid. These were stored at 4 ℃ for later use. Griess reagent was prepared by mixing equal amounts of 1% Sulphanilamide in 2.5% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. 0.5 mL

of 10 mM sodium nitroprusside in phosphate-buffered saline was mixed with 1 mL of the different concentrations of the plant extract (25−100 μg/mL) and incubated at 25 ℃ for 180 min. The extract was mixed with an equal volume of freshly prepared Griess reagent. The control samples without the extract with an equal amount of buffer were prepared similarly as was done for the test sample. The control tubes contained the extract at the same concentrations with no sodium nitroprusside. A volume of 150 µL of the reaction mixture was transferred to a 96-well plate. The absorbance was measured at 546 nm using a UV/VIS TG 50 plus UV-Vis Microplate reader (Molecular Devices, GA, USA) and ascorbic acid was used as the positive control. The percentage inhibition of the extract and standard was calculated and recorded. The percentage nitrite radical scavenging of the extract and ascorbic acid was calculated using the following formula: percentage nitrite radical scavenging activity

$$\text{Nitric oxide scavenged (\%)} = \frac{(\text{Acontrol} - \text{Atest}) \text{ x } 100}{\text{Acontrol}}$$

where Acontrol = absorbance of the control sample and Atest = absorbance in the presence of the samples of extracts or standards (Oyedemi et al., 2010).

Lipid Peroxidation: Ten microlitres (10 µL) of samples at different concentrations of 25, 50, 75 and 100 µg/mL or standard solution (1,1,3,3-tetramethoxypropane, TEP) and 40 µL of 20 mM phosphate buffer (pH 7.0) were added to a test tube on an ice bath. In each tube, 50 µL of 3% sodium dodecyl sulfate (SDS), 200 µL of 0.1 N HCl, 30 µL of 10% phosphotungstic acid, and 100 µL of 0.7% 2-thiobarbituric acid (TBA) were added. The tubes were firmly closed and boiled at 100°C for 30 min in a water bath. The reaction mixture was mixed with 400 µL of n-butanol and then centrifuged at 3000 rpm for 10 min. Supernatants were collected and passed through a UV/VIS spectrophotometer at a wavelength of 515 nm/555 nm.

$$\text{Lipid Perioxidation (\%)} = \frac{(\text{Acontrol} - \text{Atest}) \text{ x } 100}{\text{Acontrol}}$$

where Acontrol = absorbance of control sample and Atest = absorbance in the presence of the samples of extracts or standards (Wintola et al., 2021).

Determination of total antioxidant capacity: The potassium permanganate method was used to measure the total antioxidant capacity. The potassium permanganate solution was made by first dissolving 0.316 g of potassium permanganate with 50 mL of distilled water before diluting to 100 mL in a volumetric flask. The solution was kept in a brown reagent vial. With a standard sodium oxalate solution, the potassium permanganate solution was standardised. The workable solution was prepared by diluting the original 0.019 mol/L concentration of $KMnO_4$ solution to 0.005 mol/L.

The mixture was warmed for 30 min in a water bath at 37 ℃, and the absorbance of the mixture (optical density, OD, of 570 nm) was determined using a UV/VIS spectrophotomet (Kattamis et al., 2011; Kabir et al., 2014; Li et al., 2013; Vidhya et al., 2020)

$$\text{Total Antioxidant Capacity (\%)} = \frac{(\text{A control} - \text{A test}) \text{ x } 100}{\text{A control}}$$

Determination of phenolic content: Folin-Ciocalteu analysis was used to determine the total phenolic content (TPC). The concentration of the Folin-Ciocalteu reagent, which had been first decreased to 60% by the addition of water, was increased by adding one millilitre of the methanol extract to a test tube holding one millilitre of the reagent. The mixture was vortexed and allowed to stand for 30 min at 40 ℃ for colour to develop. A UV-Vis spectrophotometer (Jascov-530) operating at a wavelength of 765 nm was used to measure the absorbance after the addition of 2 mL of 20% (w/v) sodium carbonate and after the mixture had been in a dark environment for 30 min. The results were contrasted with the results for standardized Gallic acid. The results were expressed as milligrams of Gallic acid per gram of the dried extract (Panda et al., 2010).

Determination of total flavonoids: The aluminium chloride colourimetric technique was used to quantitatively determine the number of flavonoids present in the ethanolic extract overall. In a nutshell, 1 mL of the extract was combined with 0.1 mL of a potassium acetate solution containing 1 mg/mL in 2.8 mL of distilled water. The solution was then given 0.1 mL of 10% aluminium chloride. Using a UV-visible spectrophotometer, the absorbance was tracked and recorded at 415 nm after 30 min of incubation. Gallic acid equivalents (GAE) in milligrams per gramme of dry weight were used to express the total flavonoid content (Wintola et al., 2021).

Antibacterial activity: Bacteria isolates were cultured on selective media agar and were characterized phenotypically and molecularly for accurate identification. The bacteria isolates identified and used for this study include *Staphylococcus aureus*, *Bacillus nitratireducens, Klebsiella quasipneumoniae*, *Bacillus pumilus*, *Klebsiella pneumoniae*, *Brucella intermedia*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, and *Proteus mirabilis*.

Determination of Minimum Inhibition Concentrations (MICs): The activity of the extract and the minimum inhibitory concentration was determined by the serial tube dilution technique. The crude extract, the antibiotics (positive control), and saline water (negative control) were serially diluted in twofold Muller Hinton broth to obtain different concentrations. One hundred microlitres (100 µL) of standardized overnight cultured organisms were inoculated into all the test tubes except the control. The test tubes were incubated for 24 h at 37 °C and observed for any visible growth or turbidity. The lowest concentration of the plant extract in the broth which showed no turbidity was recorded as MIC (Olajuyigbe & Afolayan, 2012).

Determination of Minimum Bactericidal Concentrations (MBCs): For the determination of the MBCs, nutrient agar plates were inoculated with a loopful of culture gotten from each of the first three broth cultures that showed no growth in the MIC tubes. The MBC plates underwent a 24 h incubation period at 37 ℃, the lowest concentration of each extract that showed no bacterial growth following the incubation period was recorded as its MBC values (Olajuyigbe & Afolayan, 2012).

3. RESULTS

In this study, the ethanol extract of *L. welwitschii* was determined to indicate the potential biological activities of the plant material.

3.1 Qualitative Phytochemical Analysis

The qualitative phytochemical analysis of the ethanol extract of *L. welwitschii* revealed the presence of tannins, saponins, alkaloids, steroids, phenol, and reducing sugar as depicted in Table 1.

Quantitative phytochemical analysis: The quantitative phytochemical analysis of the ethanol extract of *L. welwitschii* is shown in Table 2. The quantitative phytochemical analysis revealed that the most prevalent phytochemical in the plant extract was tannin, which had a content of 61.88 mg/g, the phenolic content was 36.08 mg/g, and the alkaloid content was 7.45 mg/g. The concentration of steroid was 31.61 mg/g and saponin was 10.13 mg/g. Among the phytochemicals in the extract, alkaloids exhibited the lowest level at 7.45 mg/g.

Table 1. Preliminary qualitative phytochemical screening of *L. welwitschii*

s/no.	Secondary Metabolites	Ethanolic Extract
1.	Alkaloids	+
2.	Saponins	+
3.	Tannins	+
4.	Phlobatannins	+
5.	Steroids	+
6.	Cardiac glycosides	+
7.	Phenol	+
8.	Flavonoids	+
9.	Reducing Sugar	+
10.	Terpenoid	+

Present (+), Absent (-)

Table 2. Quantitative phytochemical screening of ethanol extract of *L. welwitschii*

S/no.	Phytochemicals	Quantity (mg/100g)
1.	Saponin	10.13
2.	Tannin	61.88
3.	Phlobatannins	11.99
4.	Flavonoid	29.18
5.	Cardiac glycoside	30.32
6.	Alkaloid	7.45
7.	Reducing sugar	20.75
8.	Terpenoid	21.95
9.	Phenol	36.08
10.	Steroid	31.61

GC-MS analysis: GC-MS analysis was performed on the *L. welwitschii* ethanol extract to identify the chemicals present. The GC-MS chromatogram of these chemical compounds is shown in Fig. 1, and Table 3 lists their chemical composition, name, and pharmacological activity in addition to their composition percentage. From the GC-MS analysis, forty-eight (48) bioactive compounds were expressed as percentages of the composition relative to the total composition. Although the bioactive compounds were present in the extract at varied concentrations, compounds with greater than or equal to 1.0% composition were identified as the prominent chemical compounds. The prominent bioactive compounds are 1,2,3-Benzenetriol (28.34%), 9,12-Octadecadienoic acid (Z, Z)- (13.30%), 9-

Octadecenoic acid, (E)- (12.62%), n-Hexadecanoic acid (12.00%), Catechol (4.04%), 2-methoxyphenol (2.59%), and beta-Sitosterol (2.41%). It has been noted that the bioactive chemicals' pharmacological activity are significant for medicinal purposes, while s-Indacen-1 (2H)-one, and 3,5,6,7-tetrahydro-3,3,4 (0.44%) with lower composition percentage having no reported pharmacological activities are as shown in Table 3.

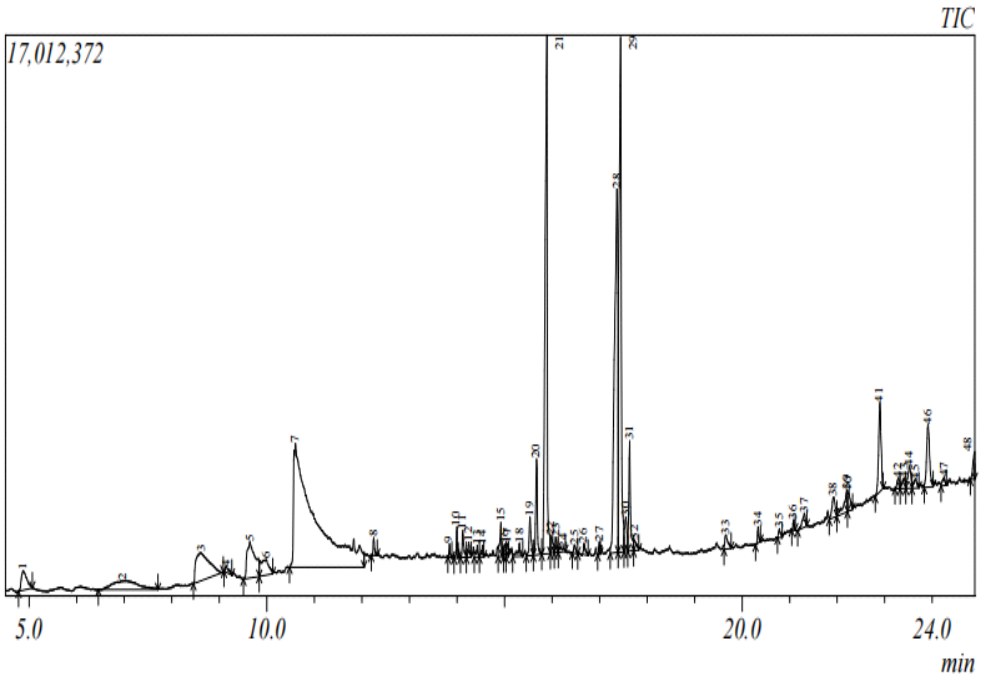


Fig. 1. GC-MS chromatograph of chemical compounds in stem bark ethanol extract of *L. welwitschii*

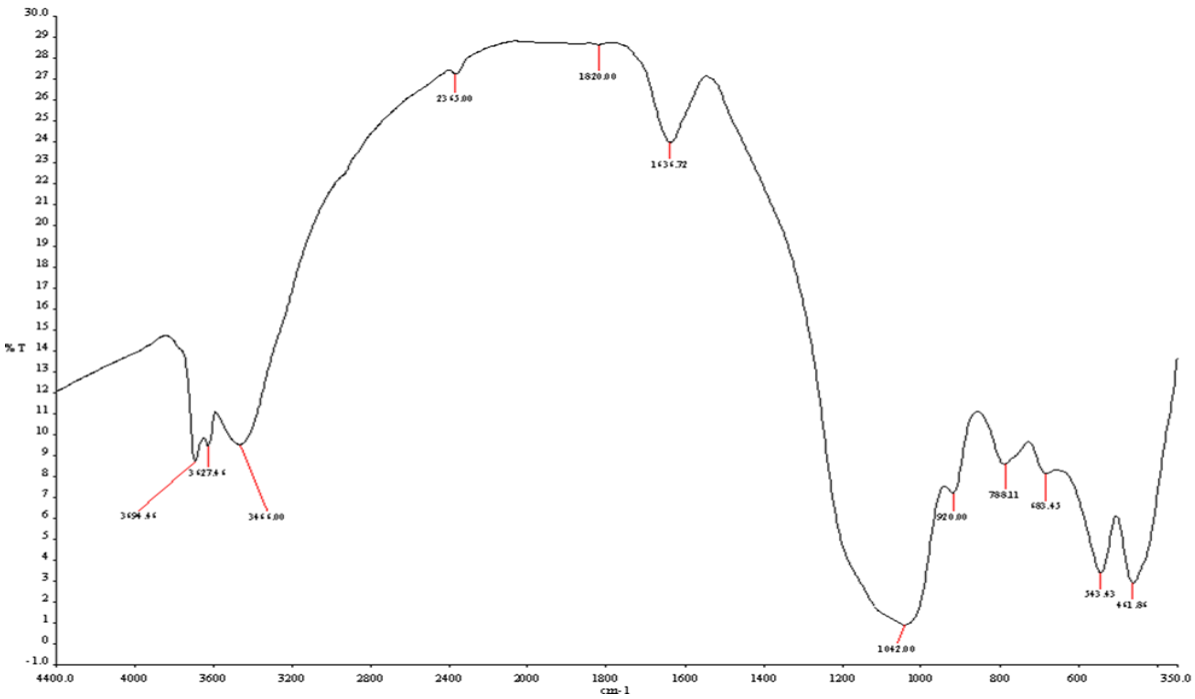
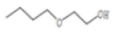
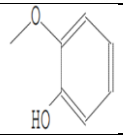
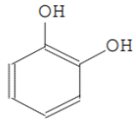
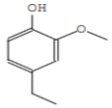
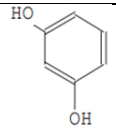
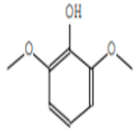
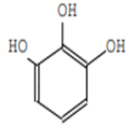
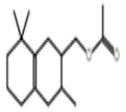
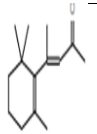
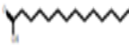
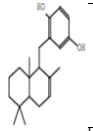
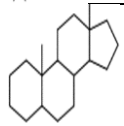
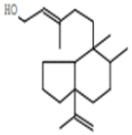
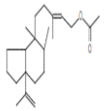
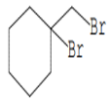
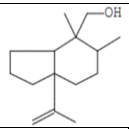
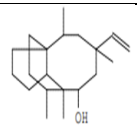
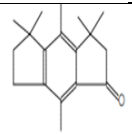
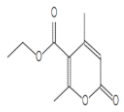


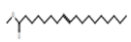
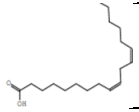
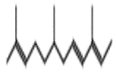
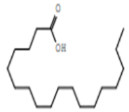
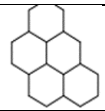
Fig. 2. FTIR spectroscopic micrograph of ethanol extract of *Lannea welwitschii*

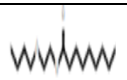
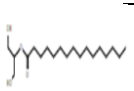
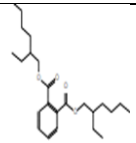
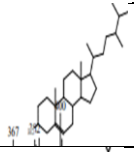
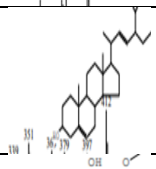
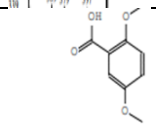
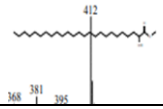
Table 3. Chemical Structure and pharmacological properties/activities of the bioactive compounds identified in ethanol extract of *L. welwitschii* by GC-MS

S/No	Peak name	Molecular formula	Molecular weight	Retention time	Peak area%	Chemical Structure	Chemico-Pharmacological Properties
1.	2-butoxyethanol	$C_6H_{14}O_2$	118	4.875	1.15		Antimicrobial activity (Salem et al., 2013)
2.	2-methoxyphenol	$C_7H_8O_2$	124	6.983	2.59		Anti-Infective Agents, Antioxidant (Kabuto et al., 2004)
3.	Catechol	$C_6H_6O_2$	110	8.617	4.04		Antioxidant activity (Rajamanikandan et al., 2022)
4.	Phenol, 4-ethyl-2-methoxy-	$C_9H_{12}O_2$	152	9.146	0.19		Antimicrobial activity (Nisha et al., 2010)
5.	Resorcinol	$C_6H_6O_2$	110	9.642	3.14		Antiseptic/Disinfectant, Anti-thyroidal activity (DeBoer et al., 2014)
6.	2,6-dimethoxyphenol	$C_8H_{10}O_3$	154	9.983	1.39		Antioxidant, Antibacterial activity (Subramanian et al., 2010)
7.	1,2,3-Benzenetriol	$C_6H_6O_3$	126	10.600	28.34		Anti-bacterial, anti-infective (Nithyadevi et al., 2015).
8.	Dodecanoic acid	$C_{12}H_{24}O_2$	200	12.250	0.24		Antimicrobial, Anticancer activity (Roger et al., 2015)

S/No	Peak name	Molecular formula	Molecular weight	Retention time	Peak area%	Chemical Structure	Chemico-Pharmacological Properties
9.	Acetic acid, (1,2,3,4,5,6,7,8-octahydro-3,8,8-	$C_{16}H_{26}O_2$	250	13.842	0.17		Antibacterial activity (Awwad et al., 2013)
10.	1-Cyclohexene, 1,3,3-trimethyl-2-(1-methylbu)	$C_{14}H_{22}O$	206	14.000	0.38		Antioxidant activity (Sivakumar et al., 2011)
11.	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	14.125	0.52		Antioxidant, Antibacterial activity (Zuzarte et al., 2015)
12.	1,4-Benzenediol,2-{1,4,4a,5,6,7,8,8a-octahydro	$C_{21}H_{30}O_2$	314	14.250	0.30		Antimicrobial activity (Amiranashvili et al., 2020)
13.	Androstane, (5. beta.)-	$C_{19}H_{32}$	260	14.425	0.22		Antimicrobial activity, Antifungal activity (Pivetta et al., 2018)
14.	5-(7a-Isopropenyl-4,5-dimethyl-dimethyl-octahydroinden	$C_{20}H_{34}O$	290	14.533	0.19		Antioxidant, Anti-inflammatory, Anti-fungal (Shahbazi et al., 2019)
15.	5-(1-Isopropenyl-4,5-dimethylbicyclo {4.3.0}	$C_{22}H_{36}O_2$	332	14.925	0.59		Antioxidant, wound healing properties, Antimutagenic properties (Shahbazi et al., 2019).
16.	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	15.000	0.20		Antibacterial activity, Antifungal activity (Ghazala et al., 2009)

S/No	Peak name	Molecular formula	Molecular weight	Retention time	Peak area%	Chemical Structure	Chemico-Pharmacological Properties
17.	1-Bromo-1-bromomethyl-cyclohexane	$C_7H_{12}Br_2$	254	15.050	0.20		Antifungal and Antibacterial activity (Baroroh et al., 2023)
18.	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)	$C_{15}H_{26}O$	222	15.317	0.35		Anti-viral activity (Chua et al., 2021).
19.	Dodecanoic acid, 10-methyl-methyl ester	$C_{14}H_{28}O_2$	228	15.533	0.81		Antimicrobial activity (Roger et al., 2015).
20.	2,4,7,14-Tetramethyl-4-vinyl-tricyclo	$C_{20}H_{34}O$	290	15.675	1.68		Anticancer activity, Antibacterial activity, Anti-inflammatory activity (Waly et al., 2015)
21.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	15.892	12.00		Anti-inflammatory activity, Antibacterial activity, antifungal activity (Gao et al., 2018)
22.	s-Indacen-1 (2H)-one, 3,5,6,7-tetrahydro-3,3,4	$C_{18}H_{24}O$	256	15.992	0.44		No activity reported
23.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	16.092	0.30		Antimicrobial, Antioxidant and Anticancer activities (Zakaria et al., 2012)
24.	2H-Pyran-5-carboxylic acid, 4,6-dimethyl-2-oxo	$C_{10}H_{12}O_4$	196	16.250	0.18		Antibronchial and Antimalarial activity (Wei et al., 2011)

S/No	Peak name	Molecular formula	Molecular weight	Retention time	Peak area%	Chemical Structure	Chemico-Pharmacological Properties
25.	n-Tridecan-1-ol	$C_{13}H_{28}O$	200	16.467	0.19		Antibacterial activity (Skinner et al., 2013)
26.	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	16.675	0.24		Anticancer activity (Saravanakumar et al., 2015)
27.	8-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	16.992	0.17		Antimicrobial activity (Adnan et al., 2019).
28.	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280	17.367	13.30		Antioxidant and antimicrobial activities (Kabir et al., 2014)
29.	9-Octadecenoic acid, (E)-	$C_{18}H34O_2$	282	17.442	12.62		Antioxidant activity (Kabir et al., 2014)
30.	Caparratriene	$C_{15}H_{26}$	206	17.550	0.84		Anti-inflammatory activity, Anti-tumor effect, Anti-proliferative activity (Li et al., 2013)
31.	Octadecanoic acid	$C_{18}H_{36}O_2$	284	17.633	2.02		Antioxidant activity (Gao et al., 2018)
32.	Octadecahydro-benzo[ed}pyrene	$C_{19}H_{30}$	258	17.733	0.31		Anticancer activity (Vidhya et al., 2020)
33.	2-Methylenetricyclo {4.3.1.0(3,8) dec-4-ene	$C_{11}H_{14}$	146	19.658	0.47		Anticancer, Anti-malarial, Anti-inflammatory, Anti-amaebic activity (Mohiuddin et al., 2022)

S/No	Peak name	Molecular formula	Molecular weight	Retention time	Peak area%	Chemical Structure	Chemico-Pharmacological Properties
34.	2-Butoxyethyl nonanoate	$C_{15}H_{30}O_3$	258	20.333	0.24		Anti-bacterial, Anti-inflammatory, Anti-cancer (Rajkumar et al., 2018).
35.	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)	$C_{19}H_{38}O_4$	330	20.783	0.21		Antioxidant activity, Wound healing activity (Habib et al., 2009)
36.	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	21.083	0.13		Antimicrobial activity, Cytotoxic activity (Yoshida et al., 2003)
37.	Campesterol	$C_{28}H_{48}O$	400	21.308	0.47		Antioxidant activity (Gupta et al., 2021)
38.	Stigmasterol	$C_{29}H_{48}O$	412	21.917	0.75		Anti-inflammatory activity, antimicrobial activity, Antioxidant activity, Anticancer activity (Gupta et al., 2021)
39.	2,5-Dimethoxybenzoic acid	$C_9H_{10}O_4$	182	22.200	0.90		Antifungal activity and antimicrobial activity (Nzogong et al., 2018)
40.	Methyl 2-hydroxy-pentacosanoate	$C_{26}H_{52}O_3$	412	22.242	0.55		Antioxidant activity (Elbadrawy et al., 2016).

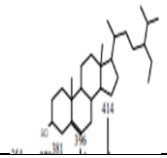
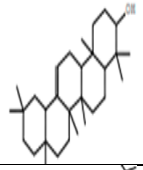
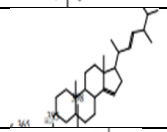
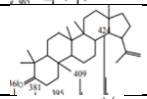
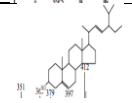
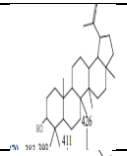
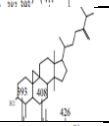
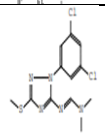
S/No	Peak name	Molecular formula	Molecular weight	Retention time	Peak area%	Chemical Structure	Chemico-Pharmacological Properties
41.	. beta-Sitosterol	$C_{29}H_{50}O$	414	22.900	2.41		Antimicrobial, anti-inflammatory, anti-cancer, antifertility, antioxidant, antidiabetic (Ambayade et al., 2014)
42.	. beta. -Amyrin	$C_{30}H_{50}O$	426	23.283	0.30		Anti-inflammatory, anticonvulsant, antidepressive, antipancreatitic, and antihyperglycemic (Nogueira et al., 2019)
43.	Ergosta-14,22-dien-3-ol, (3. beta.,5. alpha.,22E	$C_{28}H_{46}O$	398	23.400	0.36		Antibacterial, Antifungal activity (Al-Jawad et al., 2017)
44.	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424	23.525	0.75		Anti-neuroinflammatory activity (Xiao et al., 2020).
45.	Stigmasterol	$C_{29}H_{48}O$	412	23.650	0.35		Anticancer, Anti-inflammatory, Anti-osteoarthritis, Anti-diabetic, Anti-microbial activity (Gupta et al., 2021).
46.	Lupeol	$C_{30}H_{50}O$	426	23.908	2.05		Anticancer, Antiprotozoal, Anti-inflammatory, Antimicrobial, Anti-proliferative, Anti-invasive, Anti-angiogenic (Saad et al., 2022)
47.	9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl	$C_{30}H_{50}O$	426	24.250	0.24		Antioxidant activity (Sharma et al., 2020)
48.	1,2,4-1H-Triazole,1-(3,5-dichlorophenyl)-5-d	$C_{12}H_{13}C_{12}N_5S$	329	24.883	0.54		Antifungal, Anti-inflammatory, antioxidant, anticonvulsant, anticancer, antimicrobial activity (Kandasamy et al., 2014)

Table 4. FTIR Spectral Peak Values and Functional Groups of Ethanol Extract of *L. welwitschii*

S/N	Peak Values	Functional Groups	Compounds
1.	3694.46	O-H	Alcohol
2.	3627.46	O-H	Alcohol
3.	3466.00	O-H	Alcohol
4.	2365.00	O=C=O	Carbon dioxide
5.	1820.00	C=O	Anhydride
6.	1636.72	C=C	Alkene
7.	1042.00	CO-O-CO	Anhydride
8.	920.00	C=C	Alkene
9.	788.11	C=C	Alkene
10.	683.45	C-Br	Halo compound
11.	543.43	C-H	monosubstituted benzene derivative
12.	461.86	C-H	monosubstituted benzene derivative

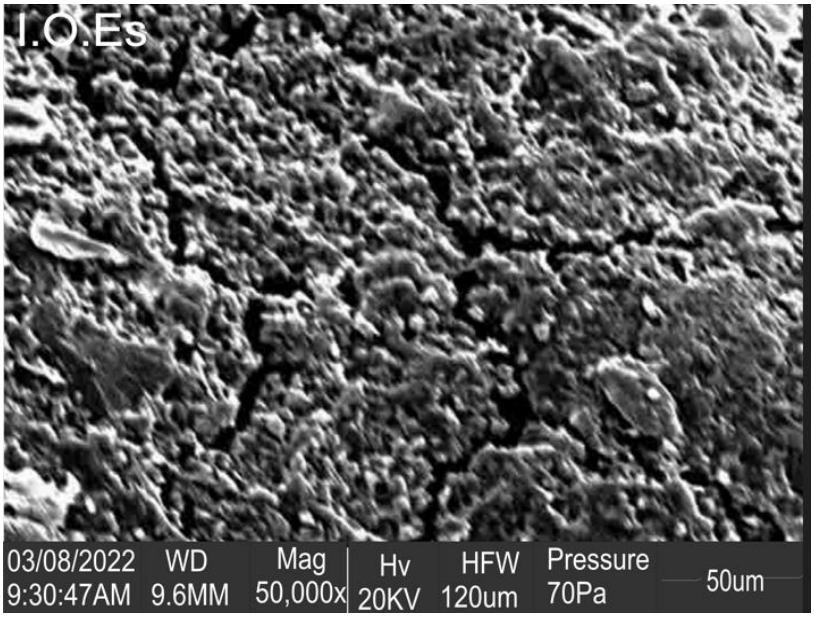


Fig. 3. SEM image of ethanol extract of *L. welwitschii*

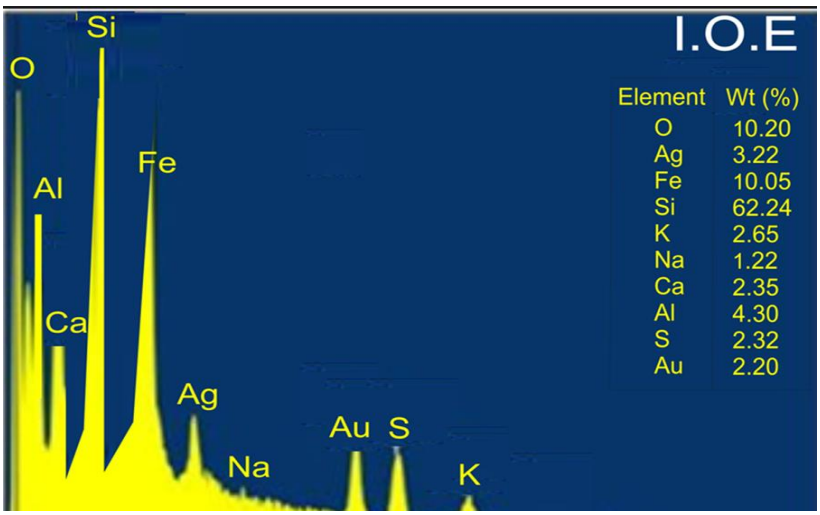


Fig. 4. EDX spectra of ethanol crude extract of *L. welwitschii*

FTIR analysis: FTIR analysis was carried out to identify the chemical bonds or functional groups of the biomolecules in the plant extract. The bonds were determined by interpreting the infrared absorption spectra. The FTIR spectra showed different absorption bands ranging from 4000 to 350 cm^{-1} which indicated the presence of some active functional groups as shown in Table 4. These results demonstrated the presence of alcohol, alkene, halo compound, carbon dioxide.

SEM results: In the Electron microscopy studies of the ethanol extract of *L. welwitschii,* the shape and structure of the plant extract were investigated through SEM-EDX analysis. These particles were found to be highly monodisperse in size with uniform shape/morphologies being formed. The SEM images displayed that the ethanol extract are highly monodispersed in size with its morphology being spherical with number of aggregates as shown in Fig. 3. The particle size of 5 0µm was determined using an advanced software named "ImageJ" for structure analysis. The SEM-coupled detector used for the EDX analysis allowed for the identification of the elemental composition of the ethanol extract of *L. welwitschii* as shown in Fig. 4. It revealed the presence of Oxygen (10.20%), Silver (3.22%), Iron (10.05%), Silicon (62.24%), Potassium (2.65%), Sodium (1.22%), Calcium (2.35%), Aluminum (4.30%, and Sulphur (2.32%). Of these elements, silicon had the highest concentration, while silver, potassium, and sodium, on the other hand, were only in trace amounts

XRD analysis: Analysis of the structure and the crystalline size of the ethanolic extract of *L. welwitschii* were carried out by XRD. The XRD analysis of the ethanol extract of *L. welwitschii* showed diffraction peaks at 2Θ = 12.5, 26.3, 30.0, 44.1, 52.5 and 56.0 degrees which correspond to the Miller indices (220), (111), (200), (220), (311) and (222), as shown in Fig. 5.

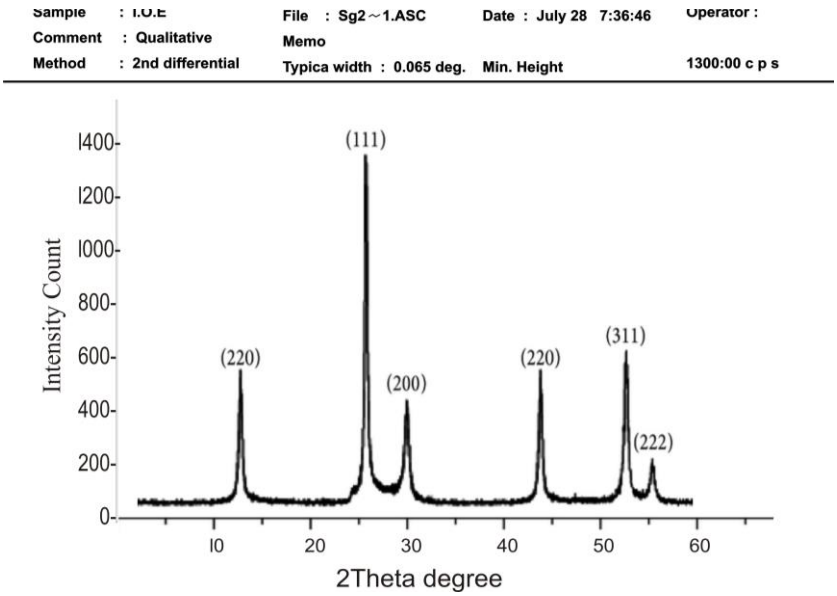


Fig. 5. XRD Pattern for ethanol extract of *Lannea welwitschii*

Table 5. MICs of *L. welwitschii* ethanol extract against tested bacterial isolates

Isolated organisms	Accession number	MIC (mg/ml)	MBC (mg/ml)
Bacillus nitratireducens	MT341782.1	0.625	0.125
Bacillus pumilus	CP027034.1	0.3125	0.3125
Bacillus pumilus	KF475865.1	0.3125	0.625
Brucella intermedia	CP061040.1	0.625	0.125
Klebsiella pneumoniae	CP027034.1	0.625	0.625
Klebsiella quasipneumoniae	CP045641.1	0.3125	0.3125
Klebsiella aerogenes	CP049600.1	0.3125	0.625
Proteus mirabilis	KU378106.1	0.625	0.625
Pseudomonas aeruginosa	MF144507.1	0.625	0.625
Staphylococcus aureus	JN092619.1	0.3125	0.3125

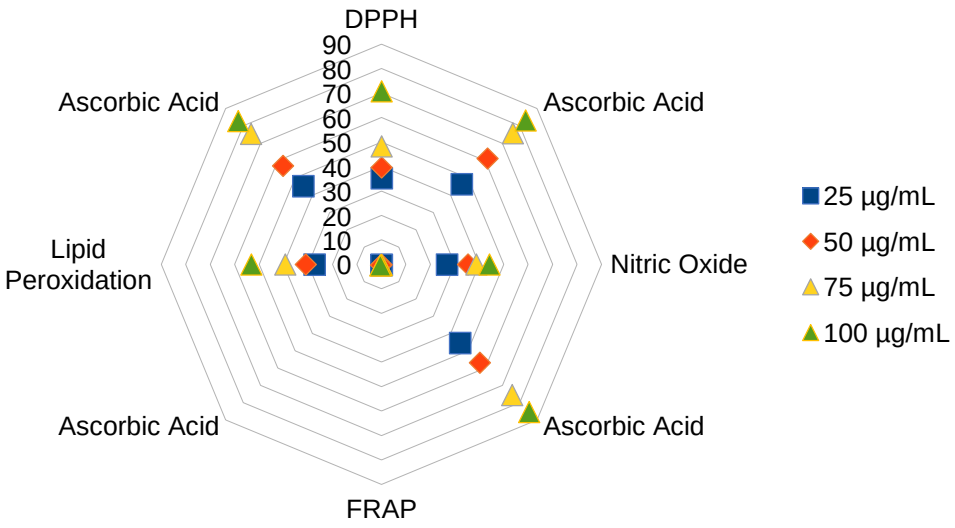


Fig. 6. Antioxidant activity of ethanol extract of *L. welwitschii*

Antioxidants activity: The ethanol extract of *L. welwitschii* antioxidant potential was performed and calculated to determine if the plant possess free radicals to destroy the effects and oxidative stress to keep the organisms safe (Srivastava & Shukla, 2015). The results revealed that as the concentration of the plant extract increased, there was an increase in the antioxidant activity of the plant extract. The extract demonstrated concentration-dependent radical scavenging activity with the DPPH radical scavenging activity being the highest, followed by lipid peroxidation activity > Nitric oxide activity > FRAP as shown in Fig. 6.

Antibacterial results: The macrobroth dilution method was used to assess the antibacterial properties of *L. welwitschii's* ethanol extract against the bacterial isolates. The result displayed the ability of the extract to suppress the growth of the bacterial isolates at varying concentrations. The MICs ranged between 0.313 mg/mL and 0.625 mg/mL. *Bacillus pumilus, K. quasipneumoniae*, *K. aerogenes*, and *S. aureus* were the most susceptible at a concentration of 0.3125 mg/mL, while *Bacillus nitratireducens, Brucella intermedia*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* had MICs value of 0.625 mg/mL as shown in Table 5.

4. DISCUSSION

"Medicinal plants are known to possess a vast array of bioactive compounds providing sources for modern medicines. The global surge in demand for plant-derived goods for medicinal and nutraceutical uses has, however, sparked the search to identify the chemical components contained in each plant and their diverse pharmacological effects" (Zakaria et al., 2012; Wei et al., 2011; Skinner et al., 2013; Saravanakumar et al., 2015; Adnan, 2019; Hassan et al., 2020). "Consequently, numerous studies have demonstrated that plant bioactive compounds may meaningfully add to the development of new and potent medications that can modify bacterial resistance as an alternate and complementary method of addressing microbial resistance" (Yaseen et al., 2015).

"Although the high prevalence of infectious diseases in tropical and developing nations has resulted in great reliance on traditional medicines due to increasingly challenging socioeconomic conditions and the unaffordability of modern medicine, antimicrobial agents derived from medicinal plants are increasingly demonstrating their potential to treat invasive infections" (Olajuyigbe et al., 2018; Kuete et al., 2011). "Additionally, as synthetic antioxidants like

Butylated Hydroxytoluene (BHT), Butylated Hydroxyanisole (BHA), propyl gallate, and tertbutyl-hydro quinine, which are known to be carcinogenic, have become more prevalent over time, it has become more important for researchers to look for safer antioxidants from natural sources" (Olajuyigbe et al., 2020). "These bioactive compounds have contributed greatly to drug production and provided compounds that could fight against various diseases and illnesses" (Altemimi et al., 2017; Atanasov et al., 2023).

"The screening of various secondary class metabolites revealed that *L. welwitschii* is a source of various phytoconstituents such as saponins, terpenoids, tannins, reducing sugars, and alkaloids proved to be responsible for various biological activities such as antimicrobial, antioxidant, and anti-inflammatory properties of medicinal plants. Although alkaloids are mainly biosynthetically derived from amino acids resulting in a variety of chemical structures mostly isolated from plants, they are known to provide unique lead compounds for medicine" (Ozdal et al., 2021). According to Bribi (Deka et al., 2013), "plants that contain alkaloids are used as analgesics, antiseptics, sedatives or anticoagulants in the treatment of coagulation disorder. Saponins represent a sizeable proportion of known plant natural products of over 200,000" (Bribi, 2018). "Saponins have a wide range of biological activities and a range of important pharmaceutical properties including anti-bacterial, anti-inflammatory, anti-fungal and anti-viral activities" (Osbourn et al., 2011). "Tannins are natural phenolic compounds that are ubiquitous in the vegetal world and can be found in fruits, woods and bark of trees. The traditional use of tannins in the medical and pharmacological industry to cure and alleviate various infections and diseases is of great potential to modern medicine and pharmacy" (Podolak et al., 2010). "These identified phytochemicals in the extract are believed to be a constituent of the defense mechanisms in plant, and be categorized as protective substances present in this plant as "phytoanticipins" and "phytoprotectants" (Pizzi, 2021).

Adeoye-Isijola et al. (2021) reported that "the combination of chromatography and mass spectrometry (GC-MS) provides the advantage of the chromatography and mass spectrometry which is the most sensitive and selective method that yields information on the molecular weight as well as the structure of the molecule". Olajuyigbe et al. (2020) also claimed that "the pharmacological activity of the methanol extract of *Ziziphus mucronata subsp. mucronata* in the production of reactive oxygen species was due to the presence of bioactive compounds in the plant extract. The forty-eight bioactive compounds identified in the ethanolic extract of *L. welwtischii* are potential novel compounds that could be isolated for therapeutic purposes as each of the compounds possessed significant pharmacologically active compounds". In the extract, the most prominent bioactive compounds with peak area percentages > 1.0 are 1,2,3-Benzenetriol (28.34), n-hexadecanoic acid (12.00), 9,12-Octadecadienoic acid (Z, Z), and 9-Octadecenoic acid, (E)- (12.62) which is a linoleic acid having hypocholesterolemic, 5-alpha reductase inhibitor, antihistaminic, insectifuge, anti-eczemic and anti-acne properties. Other bioactive compounds identified included 2-methoxy-phenol, Dodecanoic acid, Octadecanoic acid, Stigmasterol, Isopropenyl, and Lupeol. 2-methoxyphenol has the property of anti-infective agents and, antioxidant activities. According to Kabuto et al. (2004), "dodecanoic acid possess antimicrobial and anticancer activity". While stigmasterol has been shown to exert anti-angiogenic, anti-cancer, anti-inflammatory, and antimicrobial effects (Das et al., 2018), Lupeol possess anticancer, antiprotozoal, anti-microbial, anti-inflammatory, and anti-invasive activities (Nikita & Shweta, 2020). Other bioactive compounds from literature searches have been shown to possess anti-microbial, anti-convulsant, anti-cancer, anti-invasive, and other medicinal properties.

"The FTIR identified functional groups of pharmacologically active biomolecules responsible for the stabilization of the plant extracts based on the peak values produced by the FTIR spectrum. The identified functional groups included those of amines, acids, ketones, alkyl aryl ether, and halo compounds. The FTIR spectra showed peaks at 3407 cm^{-1} which was identified as N-H group of primary amines, 1712 cm^{-1} identified as C=O group of conjugated acids, 1615 cm^{-1} identified as C=C α, β-unsaturated ketone, 1252 cm^{-1} indicative of C-O alkyl aryl ether, 753 cm^{-1} indicative of C=C bending of alkenes, and 560 cm^{-1} indicative of C-I stretching of halo compound. The FTIR spectra indicated that the peaks were mainly due to the presence of different secondary metabolites present in the plant extract" (Podolak et al., 2010). "The Scanning Electron Microscopy

images indicated that the particles were spherical in shape with several aggregates having no defined morphology ranging from 40-50 nm. X-ray powder diffraction (XRD) which is an analytical technique used for the analysis of crystal structures, measuring the degree of crystallinity is rarely reported for plant extract. However, this was investigated for *L. welwitschii* to identify the phase, orientation and crystal size of the plant extract. The XRD indicated clear and distinct peaks confirming the crystalline nature of the crude ethanol extract of *L. welwitschii*. These patterns confirmed the presence of face-centered cubic crystals in the extract as evidenced by the Braggs peaks at 2Theta degree angles of 12.5, 26.3, 30.0, 44.1, 52.5 and 56.0 degrees which correspond to the Miller indices" (220), (111), (200), (220), (311) and (222), respectively for the ethanol extract of *L. welwitschii***.**

The antioxidant activities of the ethanol extract of *L. welwitschii* indicated that as the concentration of the plant extract increases, the scavenging activity of 2,2-diphenyl-1-picrylhydrazyl DPPH, Ferric Reducing Antioxidant Potential (FRAP), Lipid Peroxidation Inhibitory assay, Nitric Oxide, Total Alkaloid Capacity, Total Flavonoid Capacity, and Total Phenol Capacity increase significantly in comparison with that of ascorbic acid. The values of DPPH, FRAP, LP, NO, and TAC in the ethanol crude extracts were in the range of 0.113-35.56. This result corroborates reports from Fagbemi et al. (2022) that "the antioxidant activities were higher and concentration-dependent inhibitions in DPPH, FRAP, scavenging activity assays were observed. The antioxidant activities of the extract indicated that it has the ability to scavenge radicals able to cause different diseases. However, when bacteria are exposed to this extract, bacterial membrane fatty acids undergo oxidation, resulting in the formation of lipid peroxides". "This process triggers the generation of reactive oxygen species (ROS) due to oxidative stress induced by the extract. The oxidative stress causes bacterial membrane fatty acids' oxidation, produces lipid peroxides and redox balance favoring oxidation, disrupts the electron transport chain, interferes with bacterial metabolic reactions and ultimately activate apoptotic genes and oxidative proteins, leading to bacterial apoptosis" (Olajuyigbe et al., 2020).

"Furthermore, the antibacterial assay showed that the extract had antibacterial effects against both Gram-negative and Gram-positive bacteria. The difference in the susceptibility of the bacterial isolates could be attributed to the fact that bioactive compounds enter the thin layer of peptidoglycan and abundant pores in cell wall of Gram-positive bacteria to cause membrane damage and cell death" (Polash et al., 2022). "The difference in antibacterial activity could also be due to the differences in lipid composition, gross composition of the membranes, or even specific protein complexes present on the surface of the bacteria" (Hossain et al., 2019). "While lipid peroxide was generated because bacterial fatty acids were oxidized by extracts" (Wang et al., 2017). "The reactive oxygen species produced by the extract resulted in oxidative stress which ultimately damage the proteins, DNA and other cellular macromolecules of the bacterial cells" (Hayden et al., 2012; Li et al., 2012). The oxidation of the bacterial membrane lipids changes the permeability of bacterial cells and allows the bioactive compounds with pharmacological properties in the extract to enter the cells and interact with the cytosolic proteins and DNA to kill the bacteria. Thus, while the extract can mop up reactive oxygen species, its ability to generate reactive oxygen species in bacteria significantly contributes to bacteria death.

5. CONCLUSION

In conclusion, pharmacologically active compounds, and the functional groups in ethanol extract of *L. welwitschii* were identified. The antioxidant and antibacterial activities of this extract indicated its therapeutic potential in the treatment of bacteria involved in lower respiratory tract infections for which this plant has been used in folkloric medicine. The study, therefore, justifies the use of *L. welwitschii* in the treatment of lower respiratory tract infections in traditional and alternative medicine. Further research employing sophisticated experimental models and comprehensive toxicity assessments is necessary prior to the isolation and characterization of novel compounds from this plant which is ongoing in our laboratory.

Implication for health policy/ practice/research/medical education: The *L. welwitschii* crude ethanol extract demonstrated significant radical scavenging potentials and antibacterial activities against a variety of pathogenic microorganisms. An *in vivo* experiment could be added to this investigation to gather solid evidence of *L. welwitschii's* ethanol extract's efficacy in a biological setting.

The results may be applied to the synthesis of antibiotics and as a substitute for herbal medicine in the treatment of oxidative stress-related illnesses.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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