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Antimicrobial Effect of the Methanolic Extract of the Heartwood of *Gliricidia sepium* (jacq.) kunth ex Walp.

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Abstract

Gliricidia sepium is a multipurpose tree with sources as one of the herbal medicines in the primary health care sector, which is useful in various ways. This study assessed the chemical constituents present in the crude heartwood extract of *G. sepium* using Fourier transfer infrared spectroscopy (FTIR) with special emphasis on its antibacterial activity. Methanolic extract of the heartwood of *G. sepium* was evaluated against two gram-negative bacteria strains (*Escherichia coli* and *Pseudomonas aeroginosa*) and two gram-positive bacteria strains (*Staphyloccocus aureus* and *Bacillus sp*) using the agar diffusion method. The extract showed significant activity on all the tested bacteria; however, the activity of the extract was found to be highest against *Pseudomonas aeruginosa* (27-33mm) and *Staphylococcus aureus* (23-28mm). Although the methanolic heartwood extract activity on *E. coli* and *Bacillus cereus* was comparable with the standard antibiotics screened under similar conditions. In contrast, the activity of the heartwood extract was found to be higher than that of the standard antibiotics on *Pseudomonas aeruginosa*. The heartwood extracts of *G. sepium* were found to have high antibacterial activity over the standard antibiotics used.

Keywords: antibacterial activity, antibiotic resistance, herbal medicine, plant-based medicines, multi-drug-resistant microorganisms

Introduction

A plant whose part/parts can be used as curative or preventive measures against microbial, inflammatory, infectious, and other forms of diseases is regarded as a medicinal species (Akharaiyi *et al*., 2012; Bouayed *et al.*, 2008; Akroum *et al*., 2009). The quest for discovering new phytochemicals or secondary metabolites in plants that will serve as protection for human consumers is very much ongoing; hence, this study (Neethu and Neethu, 2016). Most developing countries and low-income persons dwelling in villages and often remote areas use medicinal plants ingested as decoctions, concoctions, and juice preparations to treat various forms of diseases and infections (Gonzales, 1980 in Rojas *et al*., 2006). Medicinal plants have a long history of use in developing and developed countries. Over 80% of the world's population relies mainly on traditional therapies using plant extracts and their active substances (WHO, 1993). Antibacterial constituents of medicinal plants and their use to treat microbial infections as a possible alternative to synthetic drugs to which many infectious microorganisms have become resistant seem very promising (Bari *et al*., 2010). Moreover, several studies have indicated that medicinal plants contain compounds like peptides, unsaturated fatty acids, aldehydes, flavonoids, alkaloids, essential oils, and phenols, which are significant therapeutic applications against bacteria and fungi, and viruses (Singh *et al*., 2010).

The secondary metabolites produced by plants are organic chemicals of high structural density that play different functions, including chemotherapeutic, bactericidal, bacteriostatic, and antimicrobial (Purihit *et al*., 1999). Several studies have been carried out in different nations in the last few decades to prove such efficiency. Many plants have been used, one of which is *Gliricidia sepium*. *Gliricidia sepium*, often simply referred to by its genus name Gliricidia is an important multipurpose, medium-sized leguminous tree belonging to the Fabaceae family (Rani, 2007) with a native range from Mexico to Colombia, but now widely introduced to other tropical zones (POWO, 2020). The tree grows well in acidic soils with a Ph level of 4.5-6.2. The

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tree is found on volcanic soils in its native range in Mexico and Central America. However, it can also grow on sandy, clay, and limestone soils (ACIAR, 2015). It is used for shading plantation crops, fodder, firewood, and a live fence (Lowe *et al*., 2004), intercropping, green manure, and rat poison (Elevitch, 2004). In traditional medicine, the leaves and branches of *G. sepium* (which contain triterpene and saponins) are used to manage fever in adults and children. They are also used as insecticides and treat infections caused by *Microsporum canis, Trichophyton mentagrophytes, and Neisseria gonorrohae* (Gupta,1995).

The traditional use of the various parts of the leaves, roots, and branches of *G. sepium* has been severally researched (Rojas *et al*., 2006; Abdullah *et al*., 2019; Sinha, 2013; Neethu and Neethu, 2016). However, the quest for sustainable management of available tree species forms the focus of this research in that extract from the heartwood of *G. sepium* might produce more and lasts longer than the other parts earlier used. Due to the problem associated with synthetic drugs' resistance to bacteria and fungi, efforts are being made to prepare useful drugs, preferably from medicinal plant extracts, which will have less or no side effects, low bacteria resistance, accessible, and easily affordable. Also, many biomedical practitioners have long ignored medicinal plant components because of the lack of effective dosage, which is believed to lead to the consumption of poisonous phytochemicals. Hence, the Fourier transfers infrared spectroscopy (FTIR) analysis of the methanolic extract of *G. sepium* heartwood will give insight into the available compounds present in the heartwood of the species.

Materials and Methods

Study Area

The study was carried out at the Department of Microbiology laboratory and University Central research laboratory within the University of Ibadan. At the Department of Forest Production and Products wood workshop, a log of *G. sepium* was felled within the university campus, debarked, and converted to sapwood and heartwood. The heartwood was planned to obtain wood shavings and later milled.

Sample Collection

Sawdust generated from the heartwood was sieved using 50µm mesh size to obtain uniform-sized particle, which was later weighed, air-dried at room temperature, and stored in an airtight container before future use. After determining the moisture content of the sawdust, a hundred gram (oven-dry weight equivalent) of the powdered heartwood of the *G. sepium* was packed in a thimble and inserted into the soxhlet apparatus chamber. The content was extracted with 400mL analytical grade methanol for 24 hours. The extract was then concentrated using a rotary evaporator at the multidisciplinary Central laboratory, University of Ibadan.

Test microorganisms, Culture medium, and Inoculation

The microorganisms used for antibacterial susceptibility test evaluation were obtained from Microbiology Department. It includes: *Bacillus cereus* and *Staphylococcus aureus* (Gram-positive bacteria) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). First, 38g of Muller Hinton Agar (MHA) components were diluted with one liter of distilled water and mixed thoroughly. This mixture was heated with frequent agitation and later boiled for 1 minute for complete dissolution, autoclaved at 121ºC for 15minutes, and cooled to 45ºC. *Next,* 15ml of cooled media, MHA, was aseptically poured at an angle of 45º into each of the eight plates. The poured plates were swirled in all directions to ensure a homogenized and uniform medium. The plates were then allowed to solidify after a few minutes and inverted to prevent the dropping of the steam formed on the lid of the plates on the solidified sample.

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Resuscitation test and Antibacterial Susceptibility test

The test organisms were resuscitated on nutrient agar from stored nutrient agar slant and incubated at 35±2ºC for 18-24 hours before use. Agar well diffusion method was used for the antimicrobial evaluations, and a 9mm core borer was used to make a well in the plate. A sterile pipette was used to introduce the different concentrations of (0%), (25%), (50%), and (100%) of the extract into the wells. The plates were made in triplicate. The standard antibiotic drugs, chloramphenicol and levofloxacin (used as positive control) were screened under similar conditions. The plates were incubated at 37ºC for 18-24 hours. The diameter of the zones of inhibition was measured in mm. The extract was classified as active as the diameter of the inhibition is similar to that of antibacterial drugs. All the assays were performed in triplicate and expressed as average values.

Spectrophotometric Analysis

FTIR is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid, or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time (Griffiths and de Hasseth 2007). For the FTIR spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 min and was filtered through Whatman No. 1 filter paper using a high-pressure vacuum pump. The extract was scanned in the wavelength ranging from 4000-500 nm. FTIR analysis was conducted using Perkin Elmer Spectrophotometer, where the characteristic peak and functional groups were detected. The wavelength of light absorbed is a salient feature of the chemical bond, as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined. (Geeethu *et al*., 2014)

Results and Discussion

Percentage Moisture Content

The initial weight of the sawdust before drying was 1,200g and was air-dried for three days at room temperature of 26.3ºC and relative humidity of 66%. The weight of the sawdust after drying was 800g. The moisture content was determined using the formula below:

$$\frac{W1}{W0} \times 100 \quad \text{...Equation 1}$$

$$\frac{1200-800}{1200} \times 100 = 33.3\%$$

The average moisture content of 33.3% showed that the heartwood sample is light, can be easily transported, and may not likely absorb water from its environment during storage.

Screening for Antibacterial Activities

The methanol extract of the heartwood of *G. sepium* was tested for antibacterial activity against four bacterial strains: *B. cereus, S. aureus, E. coli,* and *P. aeruginosa* using the agar well diffusion method (Ahmed *et al*., 1998). The extract showed significant activity on all the tested bacteria; however, the activity of the extract was found to be highest against *P. aeruginosa* (27-33mm) and *S. aureus* (23-28mm), as seen in Table 1. The methanolic heartwood extract at (25%) showed pronounced activity (Table 1) against *P. aeruginosa*. This result corroborates the findings of Adamu *et al*. (2020), where the ethanolic extract of *A. indica* root had the most reaction on *P. aeruginosa* at all levels of the given concentration. Although the heartwood extract was lower in activities on *E.coli* and *B. cereus* (20-26mm and 22-26mm), respectively, the values are similar to the 17.5± 0.6 at 200mg/ml obtained for the ethanolic extract of *A. indica* root on *E. coli* (Adamu *et al*., 2020).

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Table 1. Zone of inhibition measured in (mm) of the Methanolic heartwood extract of *Gliricidia sepium*.

Concentration	0%	25%	50%	100%
Pseudomonas aeruginosa	R	31	29	26
Pseudomonas aeruginosa	R	33	32	27
Staphylococcus aureus	R	28	24	23
Staphylococcus aureus	R	24	23	24
Bacillus cereus	R	26	25	22
Bacillus cereus	R	24	25	24
E.coli	R	26	22	20
E.coli	R	25	21	22

R stands for resistance

According to Table 2, the activity of the methanolic heartwood extract on *E. coli* and *Bacillus cereus* was quite comparable with the standard antibiotics screened under similar conditions; *E. coli* was found to be resistant to the two standard antibiotics with inhibition zone lower than (10mm) according to Johnson and Case, 1995; while the activity of the heartwood extract was found to be higher than that of the standard antibiotics on *Pseudomonas aeruginosa*. This result also indicated that the varying concentration does not affect the zone of inhibition as the higher varying concentration (100%) has a low diameter zone of inhibition.

Table 2. Zone of Inhibition measured in (mm) of Standard Antibiotics.

Antibiotics	Chloramphenicol	Levofloxacin
E. coli	R	R
Staph	23	21
Bacillus	24	23
Pseudomonas	R	R

R stands for resistance

Fourier Transform Infrared Spectrophotometry (FTIR) for the Heartwood Methanol Extract

The spectrum showed the presence of the following peak: 3920-3386.4 (cm^{-1}) (OH stretching), which indicates the presence of Alcohol and phenol (Table 3) in the extract (Olusegun, 2016). This is greater than the1371.98 reported for the Alcohol in *Khaya senegalensis* stem bark (Bashir *et al*., 2020). The extracted spectrum also showed the presence of Amine group ranging from 3584.7-3506.4 (cm^{-1}), which are slightly higher than the range of 3394.15 - 3351.29 (cm^{-1}) reported for the amine group in *Khaya senegalensis* (Bashir *et al*., 2020). In Table 3, the spectrum also indicated the presence of the alkanes group ranging from 3300.8-2899.2 (cm^{-1}) (C-H stretching. This value is greater than the 2873.74 – 2932.66 (cm^{-1}) submitted for Alkanes by Bashir *et al*. (2020). 2363.2-1615.2 (cm^{-1}) (C= C stretching) showing the presence of an aromatic compound, which may include: polycarboxylic acids, alkaloid polysaccharides, proteins, etc. These compounds are potential corrosion inhibitors in many metals such as zinc (Atolaiye *et al*., 2020) due to the electron densities surrounding the functional groups of the phytochemicals, especially soluble in more polar solvent methanol (Atolaiye *et al*., 2020). Spectrum indicated the presence of Alcohol and phenol with the peak ranging from 1262.4 -1104.8 (cm^{-1}) (C-O stretching) about Olusegun (2016), peak ranging from 798.6-620.8 (cm^{-1}) showed the presence of an Alkyl halide (C-Cl stretching).

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Table 3. Possible Compounds present in ***G. sepium*** **extract as detected by Fourier Transform Infrared spectrophotometer.**

Absorbance (cm^{-1})	Functional group	Class of compound
3920	O-H	Alcohol and phenol
3860.8	O-H	Alcohol and phenol
3798.4	O-H	Alcohol and phenol
3743.2	O-H	Alcohol and phenol
3386.4	O-H	Alcohol and phenol
1262.4	C-O	Acohol and Phenol
1164.8	C-O	Alcohol and Phenol
1104.8	C-O	Alcohol and Phenol
3584.8	N-H	Amines
3506.4	N-H	Amines
1512.8	N-H	Amines
3342.4	N-H	Amide
3261.6	N-H	Amide
2899.2	C-H	Alkanes
2778.4	C-H	Alkanes
1458.4	C-H	Alkanes
1335.2	C-H	Alkanes
3300.8	C-H	Alkynes
2363.2	C=C	Alkynes
2110.4	C-H	Alkynes
1615.2	C=C	Aromatic compound
988.0	C-H	Alkenes
845.6	C-H	Alkenes
798.6	C-Cl	Alkyl halide
703.2	C-Cl	Alkyl halide
620.8	C-Cl	Alkyl halide

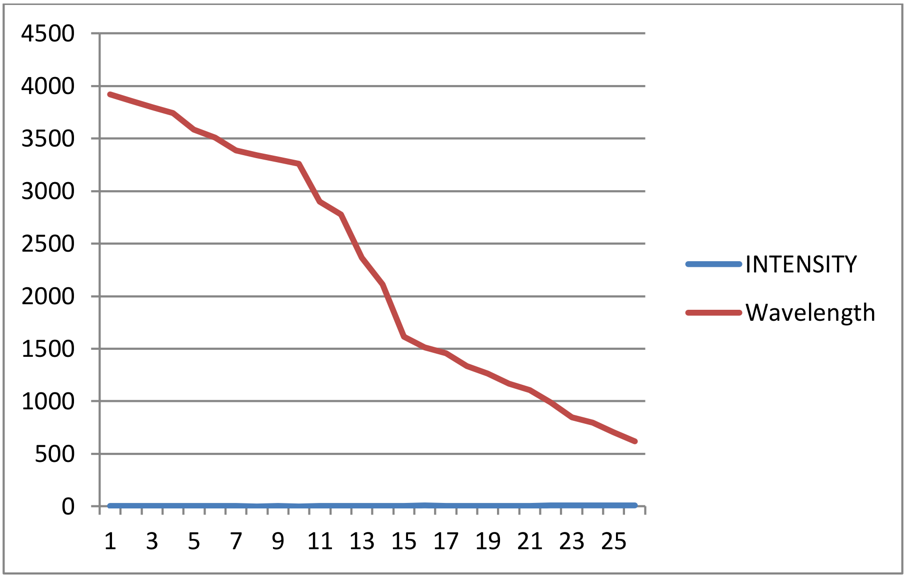

Figure 1. FTIR absorbance spectra of *Gliricidia sepium* heartwood extract.

Conclusion

The methanol extract of heartwood of *Gliricidia sepium* showed varying degrees of antibacterial activity on the four strains of bacteria tested. The variation of the susceptibility of the bacteria towards the wood extract is attributed to their intrinsic properties related to the presence of some bioactive components in *G. sepium*. Due to the emergence of antibiotic-resistant pathogens, plants are being looked upon as an excellent alternative to combat the spread of multi-drug-resistant microorganisms. From the above study, it can be inferred that the methanolic extract of *G.sepium* heartwood showed significant activity against Gram-positive and Gram-negative bacteria. Various pathogenic bacteria such as *Bacillus cereus, Escherichia coli,* and *Pseudomonas aeruginosa* have developed resistance to antibiotics. The methanolic extract of the heartwood of *G. sepium* exhibited pronounced antibacterial

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activity comparable with standard antibiotics used (Chloramphenicol and levofloxacin). The remarkable antibacterial activity exhibited by the *G. sepium* can be attributed to the synergic effect of the antimicrobial agents present in the heartwood. As the heartwood exhibited pronounced antibacterial activity comparable with standard antibiotics, it may be used as an external antiseptic in preventing and treating bacterial infections.

Conflict of Interest Statement

There is no conflict of interest reported for this work.

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