Original Research Article

ANTIBACTERIAL, PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF *GLIRICIDIA SEPIUM* CRUDE EXTRACT AGAINST HUMAN PATHOGENIC BACTERIA

Abstract

Aim:thepresent study is aimed at examine the antibacterial effect of crude extract of *Gliricidia sepium* to human pathogenic bacteria and the contents of its phytochemical and antioxidant properties.

Place and duration of the study: The study was conducted in the Department of Science Laboratory Technology, Ekiti state State university University Ado Ekiti Nigeria under ambient temperature and humidity between May and September 2023.

Methodology: the The collected plant sample was identified by Mr. Omotayo F.O. of the Department of Plant Science and Biotechnology at Ekiti State University Ado Ekiti, Ekiti State. The plant was oven dried at 61.9°C for two hours and thirty minutes, then ground into a fine powder using an electric blender after being airdried for four weeks in a cold environment. 100 g of powdered leaves were steeped in 500 ml of distilled water, N-hexane, dichloromethane, and methanol, respectively, to prepare the extracts. Following the mixes' filtration via muslin fabric. *Salmonella typhi, Staphylococcus aureus, Escherichia coli,* and *Proteus vulgaris* were obtained from Federal Teaching Hospital IdoEkitti in Ekiti State, Nigeria. The antibacterial activity of was determined using the agar diffusion method.

Results The results of the crude extract's antibacterial activity showed that the water extract had the largest zone of inhibition (12.00±1:00 mm) against *E. coli*, while N-hexane had the lowest zone of inhibition (3.33±5.77 mm) against *S. typhi*and*P. vulgaris.* Phenol content ranged from the lowest (6.19±0.01) in water extract to the highest (47.19±0.02) in DCME. The plant demonstrated good amount in the Ferric Reducing Antioxidant Power (FRAP) and DPPH scavenging assays. Methanol extract had the highest DPPH (23.53±0.03) at the lowest concentration of 100 µg/ml, and it also produced the highest DPPH (62.63±5.77) at the highest concentration of sample, 500 µg/ml. **Conclusion:** The present work highlights the possible use of *G. sepium*leaf extracts as a source of antibacterial agents that can be used to prevent human diseases.

KEYS Words; *Gliricidiasepium*, Antibiotics, Antibacterial, Antioxidants, Phytochemicals, Humans Pathogenic Bacteria, Plant extract.

1.0 INTRODUCTION

Man, always been surrounded by countless microorganisms. The disease producing microbes are playing a very important role in human life. Pathogenic microorganisms are always

trying to develop resistance to the various antimicrobial agents used for their control[1]. Consequently, treating infectious disorders with chemotherapy has proven to be an ongoing challenge. Researchers are always looking for novel antimicrobial medicines to combat the germs' growing prevalence. Therefore, it is crucial that microbiologists create new resistant strains. Therefore, medicinal plants are gifts of nature to cure limitless number of diseases among human beings[2].

Finding alternatives for antimicrobials is one of the solutions that medical authorities and researchers investigate. Nature, and more especially plants, is a significant and alluring source for novel antibacterial chemicals. Since ancient civilizations, people have used plants for medicinal purposes. Humans have learned about these plants' therapeutic potential through trial and error and the passing of knowledge from one generation to the next [3].

According to the World Health Organization, 80% of people on the planet are dependent on traditional medicine, which mostly uses plant extracts. This underscores the significance of substances obtained from plants. According to research, chemicals originating from plants also seem to have fewer harmful side effects, which is why the medical community is paying them more attention [4]. A store of unique bioactive compounds is produced when the abundant variety of therapeutic plants is paired with contemporary methods of extracting their active ingredients. Widely prevalent throughout the tropics and subtropics, the plant has been compared to a Nature's Pharmacy. In Nigerian traditional medicine, it has been widely utilized as an anticancer, analgesic, anthelminthic, antiulcer, antifertility, antifilarial, antifungal, anti-inflammatory, antiviral, antimalarial, and insecticidal. Additionally, it has antimicrobial properties. [5].

The phytochemical properties of *G.~~liricidia~~sepium* extract have attracted considerable attention in the scientific community, as evidenced by multiple studies highlighting its diverse bioactive compounds. While variations may occur due to factors such as geographical location and extraction methods, the following key phytochemicals have been consistently identified in ~~*Gliricidia*~~ *G.sepium* extract [6].Numerous phytochemicals, including flavonoids, triterpenoid, saponins, stigmastanol glucoside [7~~],~~], coumarin, coumaric acid, and melilotic acid, have been isolated and characterized from various parts of this plant. ~~specific~~ Specific phytochemical properties of ~~*Gliricidia*~~ *G.sepium* extract may be subject to variations based on factors such as geographical location, soil conditions, and plant age. However, existing literature suggests the presence of certain bioactive compounds in ~~*Gliricidia*~~ *G.sepium*.Allelochemicals from ~~*Gliricidia*~~ *G.sepium* leaves were extracted, identified, and quantified using HPLC. Rastrelli isolated a new class of 12a-hydroxy rotenoids from the methanolic extract of ~~*Gliricidia*~~ *G.sepium* bark [8]. The essential oils from *G. sepium* leaves and flowers were extracted using steam distillation and quantified using GC-MS. Because synthetic chemicals have side effects, the search for new antimicrobial agents has become necessary.

Thus, the present study is aimed at examine the antibacterial effect of crude extract of *G. sepium* to human pathogenic bacteria and the contents of its phytochemical and antioxidant properties

2.0 MATERIALS AND METHODOLOGY

2.1 Area of Study

The study was conducted in the ~~department~~ Department of Science Laboratory Technology Laboratory~~at.~~ Ekiti~~state~~ State ~~university~~ University Ado Ekiti Nigeria under ambient temperature and humidity.

2.2 Plant Identification and Preparation of the sample

The plant was gathered from Ekiti State University, located in Ado Ekiti, Nigeria. Mr. Omotayo F.O. of Ekiti State University's Department of Plant Science and Biotechnology,Ado Ekiti, Ekiti State, identified and verified the plants. The study involved the careful selection of healthy plants, with the plant parts being collected fresh, air dried in a cool room for four weeks, oven dried (Pass mark medical model–no-DHG 9101. USA) at 61.9°C for two hours and thirty minutes, and then ground up in an electric blender (Marlexelectroline IS: 4250). Up until it was needed, it was stored inside foil paper at ambient temperature, 27°C.

2.3 Preparation of Crude Extracts from Dried Plant Materials

For preparation of extracts, 100 g of powdered leaves were soaked each in 500 ml of distilled water, N- Hexane, Dichloromethane, and Methanol respectively. The mixtures in different containers were kept for 72 hours in dark room. After the mixtures were filtered using a muslin cloth order to separate the active component from the residue. The extract was collected in a sterilized container of diameter 70.40mm and allow to dry. The weight of the extract and the percentage recovery of plant extract was using the formular $\frac{WB-WA}{WA} X100/1$,~~Where~~ where WB is the weight of the pulverized powder and the WA is the weight of the extract after extraction.

2.4 Test organisms

The clinical isolates both Gram positive and Gram negative was collected from the Department of Microbiology and Parasitology unit,Federal Teaching Hospital IdoEkitti, Ekiti State Nigeria. The isolates were stored at 4^0 C before used. The tests organisms include *Escherichia coli, Staphylococcus aureus*, *Proteus vulgaris*~~.~~ and *Salmonella typhi*. These organisms were collected inside the sterile bijou bottle to prevent contamination and to maintain viability, the nutrient agar into which these organisms ~~was~~ collected was prepared in the previous day before collection. Organism for this kind of experiment must not be collected on a petri-dishesto avoid contamination that can lead to false results. Both antibiotics and leaf~~ve~~ extract would be regarded as a broad spectrum if it is able to inhibit both Gram positive and negative bacteria otherwise would regard as narrow spectrum.

2.5 Preparation of Culture Media

To ensure a positive outcome for the antibacterial screening, Mueller Hinton Agar was utilized for the antibacterial test and Nutrient agar was used to preserve the isolates. Mueller Hinton is good for in

vitro antibacterial and antibiotic activities because it permits the antibiotic and plant extract to diffuse through it well, which results in an extract's or antibiotic solution's good inhibitory impact. To prepare the nutrient agar medium, 3g was suspended in 100ml of distilled water. After autoclaving the medium for 15 minutes at 121°C, it was placed into sterile Petri plates at a temperature of about 45°C.

To make Mueller Hinton Agar (MHA) medium, 9.5 g were suspended in 250 ml of distilled water. After autoclaving the medium for 15 minutes at 121°C, it was placed into sterile Petri plates at a temperature of about 45°C.

2.6 Inoculum standardization

This was done to ensure the standard inoculum integrity in terms of turbidity uniformity. The McFarland Standard is used to estimate bacterial density in most in-vitro antibacterial screening studies. The bacterial density (Bacteria colonies and water inside sterile bottle) to be poured on the Mueller Hinton agar was compared with already prepared McFarland Standard solution (Ref.)

2.7 Antimicrobial activity

Using the agar well diffusion method, the antibacterial activity of the plant extracts was ascertained. [9]. In order to allow the microorganisms to fix to the media, exactly 0.5 ml of the standardized fraction of the test organism was aseptically put into petri dishes containing Muller Hinton Agar (MHA) for bacterial isolates. The plates were then left for approximately 20 minutes. Using a sterile cork borer with a 6 mm diameter, the wells where the extracts were to be added to the plates were carefully dug, and then tiny drops of the extracts at different concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml, and 200 mg/ml) were applied.

A commercial antibiotics disc containing 10 antibiotics at varying concentrations was positioned in the center of the agar to serve as the positive control, while a plate containing the test organism and DMSO served as the negative control. The concentration at which the extracts impede the growth of test organisms and thereafter no longer cause inhibition was determined to be the minimum inhibitory concentration. After 24 hours of incubation at 37^0C, the zones of inhibition on the triplicate-prepared plates were measured. Zones of inhibition were thought to be proof positive for antibacterial activity. The average diameter in millimeters was determined by measuring the zones of inhibition at right angles to each other using a ruler.

2.8 PHYTOCHEMICAL ANALYSIS

2.8.1 Determination of Total Phenols

Folin-Ciocalteu technique [10] was used to determine this. To 125 µl of the extract, distilled water and Folin-Ciocalteu's reagent were added. The 7% sodium carbonate solution was added after the mixture had stood for six minutes. The combination was let to stand for ninety minutes. A SpectrumLab70 spectrophotometer was used to measure the absorbance at 760 nm, and the result was expressed in gallic acid equivalents (GAE). The standard was ascorbic acid [11].

2.8.2 Determination of Flavonoid

One milliliter of distilled water was used to dissolve about 0.25 grams of the extract. Next, a 5% $NaNO_2$ solution, 0.150 milliliters of recently made aluminum chloride (AlCl3), and one milliliter of 1 M NaOH solutions were added. After letting the mixture remain for five minutes, a SpectrumLab70 spectrophotometer was used to measure the absorbance at 510 nm. The outcome was given as equivalents of quercetin (QE). The standard was ascorbic acid [11].

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2.9 ANTIOXIDANT ACTIVITIES

2.9.1 DPPH scavenging assay

Follow the methods of Adam [12], an aliquot of 1 ml of 0.3 mM DPPH ethanolic solution was added to 2.5 ml of different sample concentrations and standard ascorbic acid (ranging from 100, 200, 300, 400, and 500 µg/ml), the mixture was allowed to incubate at room temperature in a dark condition for 30 minutes. The absorbance was then measured at 517 nm. A blank made of ethanol was used. A negative control is provided by 1 ml of DPPH solution (0.3 mM) with 2.5 ml of ethanol. The studied extract's scavenging efficacy was measured by the degree of DPPH decolorization, which changed from purple to yellow. Three experiments were run in triplicate (n = 3), and average values were determined. DPPH radical scavenging activity (%) = [(Abs control – Abs sample)]/(Abs control) ×100

Where, Abs control is the absorbance of DPPH radicals + methanol; Abs sample is the absorbance of DPPH radical + sample or standard.

2.9.2 Ferric Reducing Antioxidant Power (FRAP) Assay

Samples (0.5 ml) at various concentrations (100, 200, 300, 400, and 500 µg/ml) were combined with standard ascorbic acid in 0.5 ml phosphate buffer (pH 6.6) and 0.5 ml 0.1% potassium hexacyanoferrate [K3 Fe (CN)6]. The mixture was then incubated at 50°C in a water bath for 20 minutes. To stop the reaction, 0.5 ml of 10% TCA was applied after incubation. One milliliter of the solution's top part was combined with 1 milliliter of distilled water, and 0.1 milliliter of 0.01% $FeCl_3$ solution was added. After the reaction, mixture was allowed to sit at room temperature for ten minutes, the absorbance at 700 nm was measured using the proper blank solution. All tests were performed in triplicates (n = 3). Higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as a positive control. The reducing power of the extract was linearly proportional to the concentration of the sample. Phosphate buffer (PH 6.6) was used as a blank solution[13].

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3.0 RESULTS

3.1 PERCENTAGE RECOVERY OF PLANT EXTRACTS

The table (1) shown the percentage each extract recovered after drying. It was noted that dichloromethane extract has the highest percentage of 42.60g equivalent to 100g of its original weight followed by aqueous with 27.69% equivalent to 100g of its original weight.

Table1: PERCENTAGE RECOVERY OF PLANT EXTRACTS

Solvents	Initial Weight(g) powder	Extract Weight (g)	% of Extract Recovery
Dichloromethane	100	42.60	42.60
N-hexane	100	20.67	20.67
Methanol	100	26.68	26.68
Water	100	27.69	27.69

3.2 ANTIBACTERIAL ACTIVITY

Table 2 displays the antibacterial activity of the crude extract. It was found that the water extract had the largest zone of inhibition (12.00±1:00 mm) against *E. Coli*, while N-hexane had the lowest zone of inhibition (3.33±5.77 mm) against *S. typhi* and *P. vulgaris*. Regarding dichloromethane, the maximum recorded zone of inhibition was 11±3.61 mm against *S. typhi*, while the lowest recorded zone of inhibition (6.67±5.77 mm) was observed against *E. coli* and *P. vulgaris*. In methanol extract, the lowest activity (5.00±8.67 mm) was recorded against *S. typhi*, while the highest zone of inhibition (10.00±8.72 mm) was recorded against *E. coli*. For water extract, the highest zone of inhibition (12.00±1:00 mm) was recorded against *E. coli*.

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Table 2: Zone of inhibition (mm) of crude extracts of Dicloromethane, N-Hexane methanol, and aqueous (Agar well diffusion method)

EXTRACT	*E. COLI*	*S. AUREUS*	*S. TYPHI*	P. VULGARY
Dichloromethane	6.67±5.77	10.33±0.58	11±3.61	9.67±0.57
N-hexane	11.67±3:06	11.33±2.52	3.33±5.78	3.33±5.77
Methanol	10.00±8.72	4.00±6.93	9.67±1.53	10.00±1.00
Water	12.00±1:00	8.00±7.00	5.00±8.67	9.00±1.00

The zone of inhibition for a commercially manufactured antibiotic disk against the test organism is displayed in Table 3. This was done to assess how effective the leaf extract was in comparison to antibiotics that had been purified. The manufacturer's design determined the different antibiotic concentrations. With a range of 11.5±0.50 mm to 16.5±2.50 mm, every isolate was susceptible. When Streptomycin was used at a concentration of 30 µg against *P. vulgaris*, the highest zone of inhibition (16.5±2.50 mm) was observed in the overall activities, while Ampiclox showed the least amount of activity. ~~evaluating~~ Evaluating the effectiveness of every antibiotic in relation to the

test organisms. Ampiclox had the highest activity (16±0.00) against *E. coli*, while Zinnacef had the lowest activity (13±1.00).

Against the *S. aureus*antibiotics activity was more noted in Ciprofloxacin and least recorded in Pefloxacin. For *S. typhi*, Amoxicillin has the highest activity and the least activity was recorded in Erythromycin and Gentamycin respectively.

Table 3: **Zone of Inhibition (mm) of commercially made antibiotics disks against the test organisms**

ANTIBIOTICS	Conc.(µg)	*E. COLI*	*S. AUREUS*	*S. TYPHI*	*P. VULGARIS*
CPX	10	14.5±0.50	15.5±0.50	15±0.00	15.5±0.50
S	30	15±1.00	15±1.00	14.5±1.5	16.5±2.50
SXT	30	14.5±0.50	15±1.00	14±1.00	12.5±1.50
E	10	14.5±0.50	14.5±0.50	13±1.00	14.5±0.50
PEF	10	15.5±0.50	12.5±0.50	13.5±1.50	15.5±0.50
CN	10	15±0.00	13.5±0.50	13±1.00	15.5±0.50
APX	30	16±0.00	15±1.00	14.5±2.5	11.5±0.50
Z	20	13±1.00	13.5±2.50	14.5±0.5	12.5±0.50
AM	30	15±0.00	13.5±1.50	16±0.00	15±0.00
R	25	15.5±0.50	14.5±0.50	14.5±0.5	13.5±0.50

Experimental results (mean ± SD, *n* = 3) of *G. sepium* leaf extract in mm

KEYS: CPX= Ciprofloxacin**S=** Streptomycin**SXT=** Streptomycin**E=** Erythromycin**PEF=** Pefloxacin**CN=** Gentamycin**APX=**Ampiclox, **Z=**Zinnacef**AM=** Amoxicillin **R=** Rocephin

3.3 PHYTOCHEMICAL RESULTS

The results of *Gliricidiasepium*'s quantitative phytochemical activities were displayed in Tables 4 and 5. The plant's total phenolic and total flavonoid levels both showed high quality. It worthy of note that the quantity of phytochemicals results increased with increase in concentration in all the solvents used. Table 4 shows that the maximum amount of total phenolic is found in methanol extract at the lowest concentration, followed by dichloromethane extract (DCME). However, DCME produced a considerable quantity of total phenolic compounds as the concentration increased. In general, DCME had the highest phenol concentration (47.19±0.02), whereas water extract had the lowest (6.19±0.01) level. DCME contains the maximum quantity (49.93±0.08) of total flavonoids in the table

at the lowest concentration, followed by n-Hexane Extract. However, N-hexane produced a significant quantity (98.30±0.03) of total flavonoid contents when the concentration increased to 500 µg/ml. In general, N-hexane extract had the maximum output of flavonoids (98.30±0.03), whereas water extract had the lowest level (2.09±0.01).

Table 4: Total phenolics (mg GAE/g)

Conc. (µg/ml)	Methanol Extract	n-Hexane Extract	Water Extract	Dichloromethane Extract
100	16.99±0.02	6.41±0.00	6.19±0.01	11.65±0.02
200	20.57±0.03	12.40±1.75	10.24±0.24	27.13±0.03
300	20.60±0.02	16.40±0.02	16.21±0.02	35.41±0.01
400	23.12±0.04	19.22±0.02	24.29±0.03	38.31±0.02
500	33.91±0.05	23.48±0.03	29.32±1.17	47.19±0.02
IC50	86.10±1.74	81.21±0.00	92±0.00	80.26±0.00

Experimental results (mean ± SD, *n* = 3) of *G.sepium* leaf extract in mm

Table 5: Total flavonoids (mg QE/g)

Conc. (µg/ml)	Methanol Extract	n-Hexane Extract	Water Extract	Dichloromethane Extract
100	4.58±0.02	50.56±0.12	2.09±0.01	49.93±0.08
200	10.19±0.03	56.20±0.03	5.22±0.02	58.39±0.03
300	18.62±0.03	71.18±0.03	7.95±0.09	71.28±0.02
400	20.14±0.01	82.55±0.02	13.34±0.14	80.01±0.02
500	27.38±0.02	98.30±0.03	16.21±0.01	86.79±0.03
IC50	38.71±0.00	110.65±1.74	81.42±0.00	97.63±0.23

Experimental results (mean ± SD, *n* = 3) of *G sepium* leaf extract in mm

3.4 ANTIOXIDANTS RESULTS

The results of *Gliricidiasepium*'s quantitative antioxidant activities were displayed in Tables 6 and 7. The plant demonstrated good amount in the Ferric Reducing Antioxidant Power (FRAP) and DPPH scavenging assays. Table 6 shows that the methanol extract had the highest DPPH (23.53±0.03) at the lowest concentration of 100 µg/ml. Additionally, when the sample concentration increased to 500 µg/ml, the methanol extract also produced the maximum DPPH scavenging ability (62.63±5.77). In n-hexane extract, the lowest overall activity (17.23±0.03) was observed. Table 7 reported Ferric Reducing Antioxidant Power (FRAP). At 100 µg/ml, water extract had the least activity (10.67±0.03), while the maximum concentration yielded the highest total FRAP activity (78.30±0.10).

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Table 6: DPPH scavenging assay

DPPH					Standard
Conc. (µg/ml)	**Methanol Extract**	**n-Hexane Extract**	**Water Extract**	**Dichloromethane Extract**	**Ascorbic acid for DPPH**
100	23.53±0.03	17.23±0.03	19.76±0.04	20.60±0.01	93.26±0.02
200	31.04±0.03	31.54±0.04	22.56±0.04	20.57±0.03	93.26±0.02
300	42.20±0.02	43.74±0.01	27.58±0.04	20.60±0.02	93.26±0.02
400	56.71±0.02	48.20±0.02	31.39±0.02	23.12±0.04	93.26±0.02
500	62.63±5.77	55.10±0.01	35.28±0.02	33.91±0.06	93.26±0.02
IC50	195.7±0.05	330.78±0.00	160.41±0.00	149.27±0.00	72.65±0.00

Experimental results (mean ± SD, *n* = 3) of *G.sepium* leaf extract in mm.

Table 7:Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP					Standard
Conc. (µg/ml)	**Methanol Extract**	**n-Hexane Extract**	**Water Extract**	**Dichloromethane Extract**	**Ascorbic acid FRAP**
100	31.23±0.02	26.29±0.02	10.67±0.03	21.65±0.02	87.51±0.01
200	42.31±1.72	31.72±0.02	15.25±0.03	33.79±0.03	87.51±0.01
300	47.88±0.03	57.21±0.01	17.54±0.03	48.42±5.51	87.51±0.01
400	61.57±0.12	73.39±0.02	20.72±0.02	56.97±0.05	87.51±0.01
500	64.28±0.02	78.30±0.10	33.91±0.01	62.18±0.02	87.51±0.01

IC50	71.2±0.00	57.20±0.02	49±0.00	72.15±0.00	63.01±0.00

Experimental results (mean ± SD, *n* = 3) of *G.sepium* leaf extract in mm.

4.0 DISCUSSION

The extract was screened for antibacterial properties by measuring the zone of inhibition using a conventional technique. Using the disc diffusion method, the extract was evaluated against four strains of harmful bacteria [9]. According to this study, *Gliricidiasepium* leaf extracts in dichloromethane, n-hexane, methanol, and water showed moderate activity against the clinical bacterial pathogens namely*P. vulgaris*, *S. typhi*, *E. coli*, and *S. aureus*, respectively. Natural medicine and plant-based treatments have taken notice of the potential antibacterial qualities of *Gliricidiasepium* extracts against both Gram positive and Gram-negative bacteria. Research indicates that these extracts' antibacterial action may be attributed to the presence of several bioactive substances, such as phenolic and flavonoids.

Escherichia coli is linked to a number of illnesses, the most common of which being gastrointestinal tract infections. Enteropathogenic (EPEC) and enterotoxigenic (ETEC) *E. coli* strains are frequent donors [14]. According to studies, phytochemicals with antibacterial activity have been found in extracts from *Gliricidiasepium*, and these substances may help to prevent the growth of *Escherichia coli*[15]. These bioactive chemicals' ability to obstruct vital functions or structures within bacterial cells is probably what gives them their potential antibacterial properties. The *E. coli* study's outcome was consistent with [16] research.

Studies have demonstrated that a range of plant extracts have significant effects on *Staphylococcus aureus*, a bacterium that causes a number of diseases in humans [17]. According to Nostro et al. [18], plant extracts frequently function by rupturing bacterial cell membranes, reducing enzyme activity, or interfering with vital cellular functions. These actions prevent *Staphylococcus aureus* from growing. These plant extracts' ability to combat microbes provides opportunities to investigate natural substitutes for antibacterial agents or additions. Compounds produced from plants have shown antibacterial activity against strains of *Staphylococcus aureus*. But, it's ~~important~~ importanceto take into account that different bacterial strains, extract quantities, and ambient factors can all affect how effective a treatment is.

In this investigation, the plant extract had an inhibitory impact on *Salmonella typhi*, the pathogen responsible for typhoid fever, a potentially fatal and systemic illness mainly spread by consuming tainted food or water. Akharaiyi et al. [17] reported a similar outcome. *Proteus vulgaris* is known to cause a variety of infections, which emphasizes the need of researching natural substances having antibacterial qualities, especially in light of the expanding problems related to antibiotic resistance. The bacteria of medicinal significance had a moderate inhibitory effect when exposed to *G. sepium* extract.

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*P. vulgaris and Salmonella typhi*are ~~to~~ known for numerous human infections, including bacteremia, skin conditions, ulcers, and infections obtained in hospitals, among other things. The *G. sepium* extract moderately inhibited both organisms. The result differs from [19] research, which

found no evidence of *P. vulgaris* action. The capacity of the bioactive chemicals to break bacterial cell membranes, block vital enzymes, or interfere with cellular processes is assumed to be the source of the antibacterial actions. The plant extract utilized in this investigation exhibits antibacterial properties against both gram-positive and gram-negative bacteria, suggesting that the plant has broad-spectrum activity that may be attributed to the combined action of many chemical components in the crude extract.The effects of commercial antibiotics on the chosen pathogenic organisms were investigated in this study. Resistance is not documented in this investigation. Due to a number of characteristics, commercial antibiotics are frequently thought to be more effective against human pathogenic bacteria than plant extracts. To guarantee effectiveness and safety for human usage, their formulas go through a rigorous testing and optimization process. Furthermore, the production of commercial antibiotics frequently takes place in controlled settings, guaranteeing uniform concentrations and constant quality. (Akharaiyiet al., [17]).

Although there are a variety of bioactive chemicals with antibacterial characteristics found in plant extracts, the quantities and efficacy of these compounds can change greatly between different plant sources and even batches. The precise action and potency attained by professionally crafted antibiotics may not always be matched by the complex variety of chemicals found in plant extracts.

The antibacterial activity of plant extracts is typically attributed to their major components; however, minor components also play an important role in making the extract as a whole more active than the combination of key components in synergism [20].

The phytochemicals found in plants that are in charge of preventing illness and enhancing health have been the subject of in-depth research to determine their effectiveness and comprehend the underlying mechanisms of their action [21]. In this work, the effect of active compounds in *G. sepium* extracts was determined using phytochemical and antioxidant analysis, with the results being correlated with the extract's potential for antibacterial activity. When applied to *Gliricidiasepium*, all of the extracts show good antioxidant and phytochemical activity. Support from earlier research demonstrated that the leaf extract's contain various chemical agents, such as phenols, flavanoids, and other elements categorized as active antimicrobial compounds, are responsible for the antibacterial activity as reported in this study [17].The results of the study support to a certain degree, the use of traditional medicinal plants in human and animal disease therapy and reinforce the concept of ethno-botanical approach in screening plants as potential sources of bioactive substances [13]

According to the present study, the extract from the *Gliricidia* plant is rich in antioxidants, and every solvent tested produced good levels of antioxidants.*Gliricidiasepium* is recognized for its flavonoid content, as reported in studies such as those conducted by Rodríguez et al., [22]. Flavonoids, known for their antioxidant properties, play a pivotal role in neutralizing free radicals and contributing to the plant's defense mechanisms. Phenolic acids, including caffeic acid and ferulic acid derivatives, have been identified in *Gliricidiasepium*. These compounds possess antioxidant properties and are associated with potential health benefitsas Chávez-Quintal et al., [23].

It is important to note that methanol extract has the ability to scavenge at various doses. The selection of a solvent becomes a crucial factor in plant extractions, influencing the makeup and quantities of the extracted chemicals. The disparities in activity between the extracts of dichloromethane, methanol, n-hexane, and water highlight the need of carefully choosing a solvent to

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produce plant extracts with unique and desired biological qualities [24]. Water is more polar than both methanol and dichloromethane extract, yet they both have high extraction capabilities. The variations in solvent polarity may be the cause of the increased antioxidant activity seen in the methanol and dichloromethane (DCM) extracts when compared to the n-hexane extract.

4.1 CONCLUSION

The present work highlights the possible use of *G. sepium*leaf extracts as a source of antibacterial agents that can be used to prevent human diseases. The study reveals that the results of extraction yield, phenol and flavonoid compounds and antibacterial tests varied depending upon the type of solvent being used. The leaves of *G. sepium*contain a considerable quantity of phenol - flavonoid compounds which were considered to be the major contributor for the antibacterial activities. Hence, it can be concluded that the leaves of *G. sepium*would direct to the establishment of some compounds that could be used to invent new and more potent antimicrobial drugs of natural origin. Therefore, future research should be addressed on the application of using *G. sepium*leaves as natural remedialed and to protect against infectious diseases.

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