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Antibacterial and Ftir Spectral Analysis of Methanolic Extract of *Gliricidia sepium* Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KJA and MKO designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author MKO read first manuscript. Authors OGA and KJA performed the statistical analysis, managed the interpretation of data of the study. Author KJA managed the literature searches. Authors KJA, AAA and BMO read and approved the final manuscript.

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ABSTRACT

Aim: The present study was carried out with the objective to analyse the methanol extracts of *Gliricidia sepium* leaves using Fourier transform infrared (FTIR) spectroscopic method.
Place and Period of Study: The study was carried out at the Department of Microbiology Federal University of Technology Akure between January 2013 and July 2014.
Methodology: The *in vitro* killing rate effect (antibacterial activities) of the extract- bioactive components was examined to see if they have inhibitory effect on the selected test organisms using pour plating method. The FTIR method was performed on a spectrophotometer system which detects different characteristic peak values associated with various functional groups present the extracts.
Results: All the isolates were susceptible to the extract with the number of the test organisms

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drastically reduced with time. The FTIR analysis of methanol extracts of *G. sepium* confirmed the presence of alcohols, aldehydes, alkanes, amide, alkenes, aromatics, carboxylic acids, ketones, esters, ethers, phenols, primary amines, Aliphatic bromo compound, Aryl disulphide and aliphatic amines compounds, which showed at different peaks. The FTIR method was performed on a spectrophotometer system, which was used to determine the characteristic peak values and their functional groups.

Conclusion: The results of the present study generated the FTIR spectrum profile for the medicinally important plants showed that the leaf extract of *G. sepium* can be used for pharmacological purpose.

Keywords: Gliricidia sepium; functional groups; peak value; spectroscopic; methanolic; extract.

1. INTRODUCTION

Plant-derived natural phytochemicals are the potential source for the treatment and prevention of a number of diseases in both man and poultry. Plants extracts act as a potential pipeline for novel bioactive molecules [1]. The rich biodiversity of medicinal plants make them a treasure house for obtaining novel compounds either themselves as drugs or pilot molecules for production of new drugs with different mechanism of action [1]. Plants extracts contain many phytochemicals or secondary metabolites that may act individually or in synergy against various diseases [2].

Gliricidia sepium, a leguminous tree belongs to the family Fabeacae and originated from Central America, is used in many tropical and sub-tropical countries [3]. *Gliricidia sepium* often referred to as Gliricidia and common names given to it in different places include: Mata Raton; cacahnanance in Honduras; Kakawate in the Philippines; Madriado in Honduras [4] and Agunmaniye in Yoruba part of Nigeria. It is a medium size leguminous tree belonging to the family Fabaceae. The tree is used in many tropical and sub-tropical countries for various purposes such as stalking, live fencing, fodder, coffee shade, firewood, green manure and rat poison [4] within few months, live fences can be grown from 1.5 m to 2.0 m stakes of G. sepium [5]. *Gliricidia sepium* is also known for its medicinal and insect repellent properties. Farmers in Latin America often wash their livestock with a paste made of crushed *G. sepium* leaves to ward off torsalos. In the Philippines, the leaves extract obtained from *G.sepium* is used to remove external parasites [4]. *G. sepium* is a fast growing species that takes advantage of slash and burn practices in its native range. In Jamaica, it was considered a weed as result of its swift propagation [2]. Gliricidia is normally used as green forage, protein supplement to low-quality tropical forages and by-products for cattle, sheep and goats. It can also be used as the sole feed in the dry season. Feeding levels have been 1-3% of body weight for cattle and goats, indicating a supplementation level of 30-100%, although a 20-40% level is more common [5]. Results from experiments with dairy cows and buffaloes reported similar or slightly increased milk yield and milk fat yield when concentrates were replaced by gliricidia forage up to about 25% of intake [6]. The effects of gliricidia forage on reproducing ruminants have been variable. [7]. In one trial, ewes supplemented with gliricidia yield a higher lamb crop with better lamb weights and had reduced ewe weight loss when compared with those not fed gliricidia [7]. In an unrelated trial, lambing results were poorer when gliricidia was fed, as a result of lower feed intake, possibly due to the insufficient adaptation to the forage. Laying chickens fed with sun-cured gliricidia at 4.5% of total diet gave good egg production, egg weight and yolk colour [5]. Diets containing up to 10% gliricidia can be fed to growing chicks without any effect on the performance and survival, but higher rates may have anti-nutritive effects [7].

This study was carried out to determine the functional groups associated with bioactive component present in *Gliricidia sepium* leaves for pharmacological purpose using FTIR.

2. MATERIALS AND METHODS

2.1 Bacterial Isolates

Four poultry bacterial strains were obtained from the Department of Microbiology Federal Institute of Industrial Research Oshodi Lagos, Nigeria. These bacterial strains include *Staphylococcus aureus* ATCC 25923, *Shigella flexneri* ATCC 12022, *Salmonella typhi* ATCC 6539, and *Escherichia coli* ATCC 8739.

2.2 Standardization of Inoculums

The inoculums were prepared from the stock cultures, which were maintained on nutrient agar slant at 4^0C and subcultured onto freshly prepared nutrient broth using a sterilized wire loop. The viability of the isolates were checked periodically by cultured on freshly prepared nutrient agar.

2.3 Collection and Preparation of Plant Sample

Gliricidia sepium plant was collected from forest in Ipinsa village of Ondo State, Nigeria. The plants were identified and authenticated by Mr. Hassan G. F. in Department of Crop Soil and Pest Management Federal University of Technology Akure, Ondo State. The leaves were then separated from the stems and air-dried two weeks at room temperature (25±2ºC). The dried leaves were pulverized by grinding machine (type N model) into smooth powder. The powder was further sieved by 1.18mm sieve.

2.4 Extraction of the Plant Sample

The solvents used for extraction was methanol. For the sample, 150 g was dissolved in 560 ml of methanol. The mixture were kept for 72 hours in a tightly sealed amber glass at room temperature, protected from sunlight and mixed several time by shaking. The mixture then filtered through muslin cloth. The liquid extracted was subjected to rotary evaporator under reduce pressure. The collected extract was allowed to dry under room temperature [8].

2.5 Quantitative Determination of the Lethal Effect of the Extract on the Bacteria Isolates

Firstly to carry out lethal dose test of the extract, the density of bacterial suspension inoculated onto the media for lethal test was determined by comparison with 0.5 McFarland standard of Barium chloride solution [9]. For the killing rate, the method of [10] was used to determine the in vitro rate of killing on the test bacteria by *G. sepium* leaf extract. One milliliter (1ml) of the extract was then thoroughly mixed with 0.5 ml of 18 hour culture suspension of the test bacteria. The suspensions were thoroughly mixed and held at room temperature (28-30^0C) and thekilling rate was determined for 0, 1, 2, 3, 4, 6, 8, 10 and 24 hours interaction of the extract with the bacteria cells. Exactly 0.1ml cell suspension was withdrawn at interval and put into sterile petri dish. Sterile nutrient agar at 45^0C was added, swirled, allowed to set and incubated at 37^0C for 24 hours. Controls were set up alongside the experiment using sterile distilled water as control.

2.6 Column Chromatography

The column (30x8 cm column) was packed using a simple dry- pack method. A 250 ml burette was plugged with a small piece of glass wool with the aid of applicator stick to tamp it down firmly. Silica gel of 60g for column chromatography was poured into the plugged burette using a 100 ml beaker; the filled column was clamped securely to a ring stand using a small 3 pronged clamp and placed on the bench top. When properly packed, the silica gel fills the column to just below the indent on the burette. This leaves a space of 5 cm on top of the adsorbent for the addition of solvent. Petroleum ether, chloroform and methanol was used to pre elute the column respectively. Petroleum ether was added to the top of silica gel to flow slowly down the column. One gram of the sample to be purified was thoroughly mixed with 60 grams silica gel, loaded onto the column fresh eluting solvent and the elution process began. 100 ml fractions were collected in round bottom flasks and distilled. The solvent was changed with chloroform and collected fractions were distilled. Lastly, the solvent was replaced with the methanol and collected fractions were distilled. More bands were observed until the bands were fully washed down with methanol [11]. The collected and distilled fraction was distributed into small tube and allowed to dry at room temperature.

2.7 Fourier Transform Infra Red Spectroscopy

Infra red (IR) analysis was done with the aid of infra red spectrophotometer (Perkin-Elmer, spectrum bx) at the Redeemer University Remo Ogun State Nigeria. A drop of purified extract was placed on fused sodium chloride (NaCl) cell. It was carefully placed on cell clamped loosely and fixed on the infra red beam. The infra red data was compared to the table of IR frequencies using the methods of [12].

3. RESULTS AND DISCUSSION

The Fig. 1 shows the killing rate of methanolic extract on the *S. aureus*, *S. flexneri*, *S. typhi*, and *E. coli* respectively. The rate of killing of the

extracts showed that there is a continuous decrease in the number of colony with respect to time though the extracts did not completely kill the organisms even at 24 hours of exposure. The ability of this extract to reduce the number of colonies with respect to time indicated that the extract has a lethal effect on the organisms. Therefore there is an indication that if there is a prolonged time of exposure the probability of total lethal effect of the extract on the organisms may be possible. This observation is in line with [10] who reported that the rate of killing of extract depend largely on the time of exposure while working on the Phytochemical and Antimicrobial properties of *Vernonia amygdalina* Del and *Vernonia tenoreana* Sensu Eyles.

Fourier transform infrared spectrometry (FTIR) is a physico-chemical analytical technique used to identify the functional groups of the bioactive components in a plant or other related materials based on the peak value in the region of infrared radiation. [12] The FTIR method measures the vibrations of bonds in chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample [12]. The bond in the compound can be determined through the interpretation of infrared spectrum absorption. With different solvent used different peak characteristic in their spectrum were observed but all pointed towards the same constituent present in the *G. sepium* leaves. The little differences in their spectrum were due to the ability of each solvent to extract different bioactive component present in the plant.

The chloroform fraction of methanol extract of *G. sepium* leaves in Table 1, Fig. 2. revealed twenty characteristics peaks. The observed absorptions were ranged from medium, strong, sharp and weak absorption bands. The weak absorption at 3412.19 cm^{-1} was assigned to N-H of aliphatic amine. The N-H band at this region was due to the presence of alkaloid in methanolic extract of *G. sepium*.

The weak but sharp absorption at 3412.19 cm^{-1} was assigned to N-H of aliphatic amine. The N-H band at this region indicates to the presence of alkaloid in extract of *G. sepium* leaves. The absorption bands at 3354.32 cm^{-1} was corresponded to the presence of O-H stretching vibration, the absorption in this region was due to the alcoholic or phenolic compounds present in the extract of *G. sepium*. The presence of O-H group in the extract of a medicinal plant has been responsible for the high antibacterial activity against microorganisms [13]. The strong absorption band at 3956.97 cm^{-1}, 2922.25 cm^{-1}, 2850.88 cm^{-1} were assigned to C-H stretching vibration. Such vibration in this region was due to the presence of alkanes and alkenes. [14] The absorptions 2800-3000 cm^{-1} were due to C-H stretching vibrations that are mainly generated by lipids [14].

The band at 1735.99 cm^{-1}, 1261.49 cm^{-1}, and 1101.39 cm^{-1} are assigned to C-O which possibly due to the presence of Esters, ketones, saturated aliphatic and Aldehydes. The presence of flavonoid was confirmed by the absorption peak 1101.39 cm^{-1} .for C-O Stretching. The presence of C=O stretch at the 1734.01 cm-1 confirms the presence of saponins type of compounds of methanolic extract of *G. sepium* leaves. Direct detection of saponins in medicinal

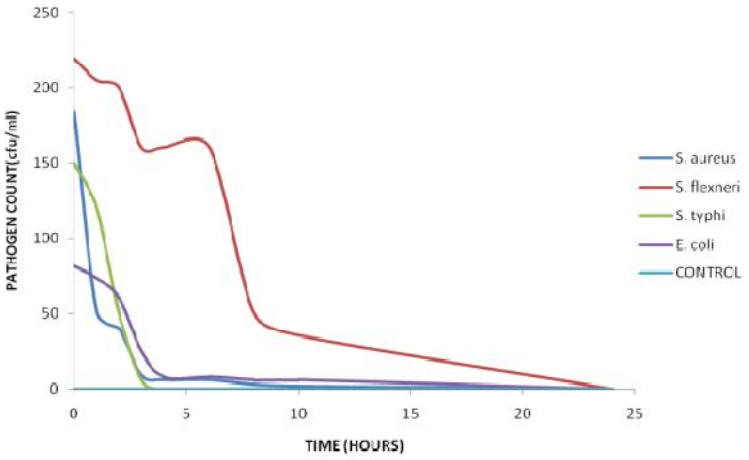

Fig. 1. Lethal rate of the extract against test organisms

plants using FTIR spectroscopy was reported earlier by [15] where characteristic peak was noted at –OH, -C=O, C-H, and C=C absorptions of oleanone triterpenoid and the C-O-C absorptions indicated glycoside linkages to the saponins [16].

The infrared spectrum at 918.15 cm^{-1} indicated C-H bending of alkanes. The absorption bands at 806.27 cm^{-1} and 719.47 cm^{-1} corresponded to C-H bending which may be possibly due to the presence of aromatic rings compound. The peak at 669.32 $cm^{-1}$was assigned to C-Br which indicates the presence of aliphatic bromo compound. The absorption band at 557.45 due to C-I was possibly as result of the presence of aliphatic iodo compound. The band 472.58 indicates the presence of S-S stretching which is polysulphides. The presence of all these organic compound are significant in the antibacterial performance of the *Gliricidia sepium* leaves [13]. There is no band in between the region 2220-2260 cm-1 indicates that no cyanide groups in all three solvents fraction and this imply that *G. sepium* does not contain any toxic substances. [13] revealed that functional groups of carboxylic acids, alcohols, aldehydes, amines, amides, sulphur derivatives, organic hydrocarbons and halogens that are responsible for various medicinal properties.

Fifteen characteristics absorptions were observed with methanol fraction (Table 2, Fig.3). The observed absorptions were ranged from strong, medium, sharp and weak absorption bands.

The strong absorption bands at 3404.47 cm^{-1} and 3348.54 cm^{-1} were corresponded to the presence of O-H stretching vibration, the absorption in this region was due to the alcoholic or phenolic compounds present in the extract of *G. sepium* leaves. The presence of O-H group in the extract of medicinal plant has been responsible their antibacterial activity against pathogens [13]. The medium absorption band at 2918.40 cm^{-1}, 2850.88 cm^{-1}, 1456.30 cm^{-1}, and 1410.01 cm^{-1} were assigned to C-H stretching vibration. Such vibration in this region was due to the presence of alkanes and alkenes groups [14].

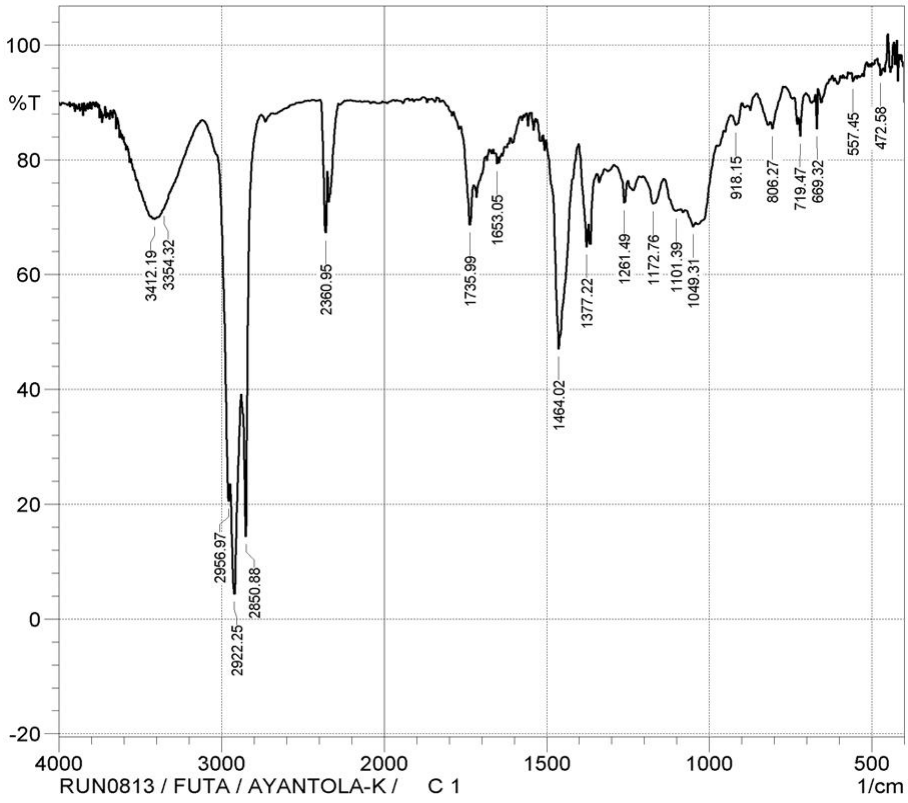

Fig. 2. FTIR Spectrum of chloroform fraction of methanolic extract of *G. sepium*

Table 1. Chloroform fraction of methanolic extract of *G. sepium*

S/N	Peak values (cm^{-1})	Bond	Functional groups assigned	Group frequency (cm^{-1})
1	3412.19	N-H Stretching	Aliphatic Amine	3300-3500
2	3354.32	O-H stretching	Alcohol/phenol	3200- 3600
3	2956.97	C-H stretching	Alkanes	2850-2970
4	2922.25	C-H stretching	Alkanes	2850-2970
5	2850.88	C-H Stretching	Alkanes	2850-2970
6	2360.95	-	Unknown	-
7	1735.99	C-H stretching	Carbonyl	1690-1760
8	1653.05	-C=C- Stretching	Alkenes	1610-1680
9	1464..02	C-H Bending	Alkanes	1340-1470
10	1377.22	C-H Bending	Alkanes	1340-1470
11	1261.49	C-O Stretching	Alcohol, Carboxylic acid, Ester and Ether	1180-1360
12	1172.76	C-O Stretching	Aliphatic amines	1050-1300
13	1101.39	C-O	Alcohol, Ether, Carboxylic acid	1050-1300
14	1049.31	C-N Stretching	Aromatic amines	1020-1250
15	918.15	O-H bending	Carboxylic	910-950
16	806.27	C-H	Aromatic rings	675-950
17	719.47	C-H	Aromatic rings	675-950
18	669.32	C-Br	Aliphatic bromo compound	500-600
19	557.45	C-I	Aliphatic iodo	
20	472.58	S-S	Polysulphide	

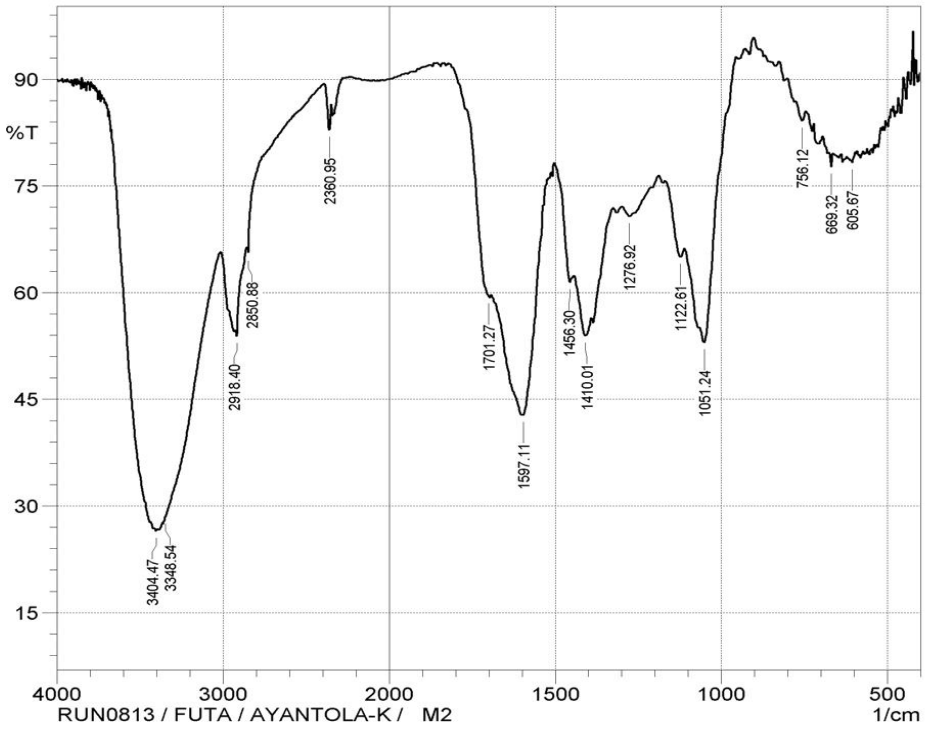

Fig. 3. FTIR Spectrum of methanol fraction of methanolic extract of *G. sepium*

The absorptions in the frequencies 2800-3000 cm^{-1} were due to C-H stretching vibrations that are mainly generated by lipids [14]. The sharp medium band at 1701.27 cm^{-1}, 1747.57 cm^{-1} and

1716.70 cm^{-1} are assigned to C-O which possibly due to the presence of Esters, ketones, saturated aliphatic and Aldehydes. The absorption bands at 1276.92 cm^{-1}, 1122.61 cm^{-1}, 1230.63 cm^{-1} and 1051 cm^{-1} stretching assigned to C-O which indicated the presence of Alcohols, Ester, Carboxylic acid and ether. The amide bands are primarily associated with stretching motion of C-O [17]. The C-O absorption is sensitive to the environment of peptide linkage and also depends on protein's overall secondary structure [18]. There is no band in between the region 2220-2260 cm-1 indicates that no cyanide groups in all three solvents fraction and this imply that *G. sepium* does not contain any toxic substances. The presence these functional groups in *Gliricidia sepium* make it a potential pharmacological source for new antibacterial agents against poultry pathogens.

Twenty three characteristics peaks observed in the petroleum ether fraction of methanol extract of *G. sepium* leaves in Table 3, Fig. 4.

The weak absorption bands at 3649.44 cm^{-1} was assigned the presence of O-H stretching vibration and the absorption in the region 3500-3650 cm^{-1} was due to the alcoholic or phenolic compounds present in the extract of plants leaves. [13]. The strong pointed absorption band at 2956.97 cm^{-1}, 2924.18 cm^{-1} and 2852.81 cm^{-1} are assigned to C-H stretching vibration. Such vibration in the region 2850-2970 cm^{-1} was due to the presence of alkanes and alkenes [14].

The absorptions in the region 2800-3000 cm^{-1} were due to C-H stretching vibrations that are mainly generated by lipids [14]. The band at 1747.57 cm^{-1} and 1716.70 cm^{-1} are assigned to C-O which possibly due to the presence of Esters, ketones, saturated aliphatic and Aldehydes. The bands at 1647.26 cm^{-1} indicated alkenes due to -C=C-. The vibration at frequencies 1500-1600 cm^{-1} indicates the presence of aromatic ring compound. The absorption bands at 1464.02 cm^{-1} and 1356.65 cm^{-1} indicated the C-H bending. The absorptions in this region were due to the presence of alkanes. The bands at 1261.49 cm^{-1}, 1230.63 cm^{-1} and 1097 cm^{-1} stretching assigned to C-O which indicated the presence of Alcohols, Ester, Carboxylic acid and ether. The IR finger prints of protein are characterized by a set of absorption bands represented as the amide region and the C-H region. The common modes in protein structure studies in the amide region are amide I, II and III. The amide I band originate principally from the C=O stretching vibration of the peptide group, while, the amide II band is fundamentally N-H bending with a contribution from C-N stretching vibrations [19.] The infrared spectrum at 918.15 cm^{-1} indicated C-H bending of alkanes.

Table 2. Methanol fraction of methanolic extract of *G. sepium*

S/N	Peak (cm^{-1})	Bond	Functional groups assigned	Group frequency (cm-1)
1	3404.47	O-H Stretching	Alcohols /phenols	3200-3600
2	3348.54	O-H Stretching	Alcohols/ Phenols	3200-3600
3	2918.4	C-H Stretching	Alkanes	2850-2970
4	2850.88	C-H Stretching	Alkanes	2850-2970
5	2360.95	-	Unknown	-
6	1701.27	C=O	Carboxylic acid, Aldehyde, Ketone.	1690-1760
7	1597.11	C-C Stretching	Aromatic rings	1500-1600
8	1456.3	C=H	Alkanes	1340-1470
9	1410.01	C-H Stretching	Alkanes	1340-1470
10	1276.92	C-O	Alcohol, Carboxylic acid, Esther, Ether.	1050-1300
11	1122.61	C-O	Alcohol, Carboxylic acid, Esther, Ether.	1050-1300
12	1051.24	C-O Stretching	Alcohol, Carboxylic acid, Esther, Ether.	1050-1300
13	756.12	C-H Stretching	Aromatic rings	690-900
14	669.32	C-Br	Aliphatic bromo compound	600-700
15	605.67	C-Br	Aliphatic bromo compound	600-700

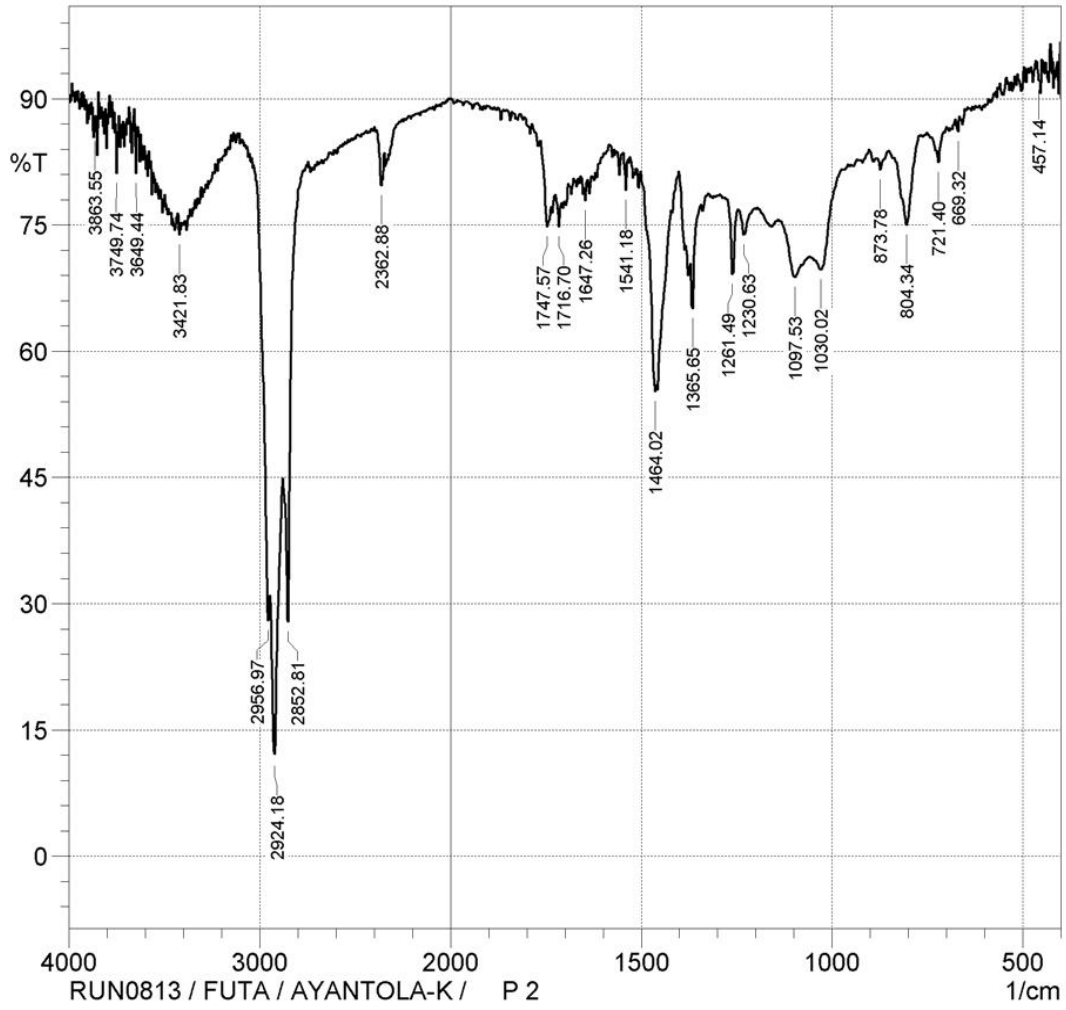

Fig. 4. FTIR Spectrum of petroleum ether fraction of methanolic extract of *G. sepium*

Table 3. Petroleum ether Fraction of methanolic extract of *G. sepium*

S/n	Peak (cm^{-1})	Bond	Functional groups assigned	Frequency group(cm^{-1})
1	3863.55	-	Unknow	-
2	3749.74	-	Unknow	-
3	3649.44	O-H	Alcohols,Phenols	3500-3650
4	3421.83	N-H	Aliphatic Amines	3300-3500
5	2956.97	C-H	Alkanes	2850-2970
6	2924.18	C-H stretching	Alkanes	2850-2970
7	2852.81	C-H stretching	Alkanes	2850-2970
8	2362.88	Unknow	Unknow	-
9	1747.57	C=O stretching	Carboxylic acid, Ester, Ether, Alcohols	1690-1760
10	1716.7	C=O stretching	Carboxylic acid, Ester, Ether, Alcohols	1690-1760
11	1647.26	-C=C- Bending	Alkenes	1610-1680
12	1541.18	C-C streching	Aromatic rings	1500-1600

S/n	Peak (cm^{-1})	Bond	Functional groups assigned	Frequency group(cm^{-1})
13	1464.02	C-H Bending	Alkanes	1340-1470
14	1356.65	C-H Bending	Alkanes	1340-1470
15	1261.49	C-O	Alcohol, Carboxylic acid, Esther, Ether.	1050-1300
16	1230.63	C-O	Alcohol, Carboxylic acid, Esther, Ether.	1050-1300
17	1097.53	C-O Stretching	Alcohol, Carboxylic acid, Esther, Ether.	1050-1300
18	1030.02	C-N	Primary amines	1020-1090
19	873.78	C-H	Aromatic rings	690-900
20	804.34	C-H	Aromatic rings	690-900
21	721.4	C-H	Aromatic rings	690-900
22	669.32	C-Br	Aliphatic bromo compound	600-700
23	457.14	S-S	Aryl disulphide	430-500

The C-N stretching due to primary amines was observed at 1030.02 cm^{-1}. The absorption bands at 873.78 cm^{-1}, 804.34 cm^{-1}, and 721.40 cm^{-1} indicated C-H bending which may be possibly due to the presence of aromatic rings compound. The peak at 669.32 cm^{-1} was assigned to C-Br which indicates the presence of aliphatic bromo compound. The S-S due to the Aryl disulphide was observed at 475.14 cm^{-1}.

[13] revealed that functional groups of carboxylic acids, alcohols, aldehydes, amines, amides, sulphur derivatives, organic hydrocarbons and halogens that are responsible for various medicinal properties. The peaks revealed the number of functional groups that have a great significant towards medicinal prospects of *G. sepium*.

4. CONCLUSION

The results obtained in this study has revealed that the methanolic extract of *Gliricidia sepium* contained a lot of chemical constituents that are of pharmacologically important in the production of antibacterial agents. By using FTIR spectrum analysis, we can confirm the functional group's presence in the extract, identify the medicinal materials from the adulterate and even evaluate the qualities of medicinal materials for pharmacological purpose.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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