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CHROMATOGRAPHIC SEPARATION OF AQUEOUS LEAF EXTRACTS OF *ACACIA NILOTICA* AND CHARACTERIZATION OF THE ACTIVE CHEMICAL COMPOUNDS FOR ANTIBACTERIAL APPLICATION

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Abstract
Medicinal plants are at great interest to drug industries, as herbal medicines and their derivative products are often prepared from crude plant extracts. The present study aimed to determine the antibacterial activity against bacterial isolates form *Acaia nilotica leaves*. The crude aqueous leaves extract of *Acacia nilotica* showed the antibacterial activity for all the bacterial isolates at three different concentrations (60, 80 and 100 mg/ml), with *E. coli* having the higher activity and *S. typhi* with lower activity for all the concentrations. Sub-fraction B3 showed the antibacterial activity for all the bacterial isolates, while sub-fraction B4 showing the antibacterial activity for only *E. coli*. Sub-fraction B1, B2 and B5 showed zero activity for all the bacterial isolates. The FT-IR analysis revealed the presence of OH, C-H, =C-H, C=O, C=C, C-O functional groups. The result of GC-MS analysis showed the presence of eleven bioactive compounds. The antibacterial activity might be due to presence of phytochemicals in the plant extract such as terpenoids, flavonoids,

phenols, glycosides, saponins, alkaloids, steroids, and tannins. Hence, the need to isolate and identified the active chemical compounds that have medicinal applications in the fraction as well as to elucidate their exact mechanism of action in different disorders.

Keywords: *Acacia nilotica*, FT-IR analysis, GC-MS analysis, biochemical compounds, leaves

1. Introduction

Medicinal plants play an important role in pharmaceutical industries, as herbal medicines and their derivative products are often prepared from crude plant extracts, which contain a complex mixture of diverse phytochemical components. Bioactive chemical compounds extracted from plants particularly higher plants have been suggested as an alternative source for antibiotics. Different plants species contain different chemical features. This approach is attractive, because they constitute a prospective source of bioactive chemical compounds that have been acknowledged by the general public as reasonably safe and often act at numerous and new target sites, thereby decreasing the potential for resistance [1]. Acacia nilotica (L.) usually well-known as Babool that belong to family Fabaceae and normally growing as average sized tree. The plant leaves are bipinnate having 3-6 pairs. The plant is stated to have distinctive medicinal applications such as antifungal, anticancer, strength and nutrient supplement, antimicrobial, stomach pain and treatment of wounds. Terpenoids, flavonoids, tannins, curcumin and phenolics are natural antioxidants that are found in this plant [2]. Extraction remain the key step for the retrieval and isolation of bioactive chemical compounds from plant materials, before constituent investigation [3]. Therefore, for the detection of lead chemical compounds to be use as healing drugs, the active biochemical compounds present in medicinal plants needs to be isolated and identified [4]. With the help of FT-IR and GC-MS methods of analysis can be serve as a motivating tool for identifying the amount of several active principles of herbal medicine.

2. MATERIAL AND METHOD

2.1 Materials

The solvent for extraction used was distilled water, the rest of reagents and chemicals were of analytical grades.

2.2 Methods

2.2.1 Collection and Identification of Sample

The fresh leaves of *Acacia nilotica* was collected in "Aliero" Kebbi State, Nigeria. The sample were identified and authenticated at the Department of Plant Science and Biotechnology Kebbi State University of Science and Technology, Aliero. The plant sample was given a Voucher number of 284. The plant sample was dried under the shade, pulverized and kept in a container till further use.

2.2.2 Preparation Plant Leaves

The fresh leaves were wash three times with tap water and once with distilled water to removed dirty and dried under the shade, grounded to fine powder using mortar and pestle. Exactly 500 g of the fine powder was mixed with 1.5 L of distilled water for about 24 hours and then filtered using Whatman filter paper number one. The concentrated filtrate was stored in clean conical flask at a temperature of about 4 ^{o}C for further analysis.

2.2.3 Phytochemical Screening

The methods described by [5-7] with slight modification were used in carrying out quantitative phytochemical screening.

2.2.4 Column Chromatography

Column chromatography was done using method describe by [8].

2.2.5 Analytical Thin Layer Chromatography (TLC)

Analytical thin layer chromatography (TLC) was done using combining methods by [9-10].

2.2.6 Test Bacterial Isolates

Three pathogenic bacteria *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi.* were isolated using a serial dilution technique on nutrient agar medium. The soil sample for the isolation of bacterial species was collected at Nasarawa Area "Aliero". 1 g of soil sample was mixed with 10 milliliters of distilled water. The soil suspension was serially diluted from 10-1 to 10-6. 0.1 milliliters of the diluted sample were pipette and spread onto nutrient agar plates. The plates were incubated at 37°C for 24 hours. The most prominent colonies were picked and streaked onto fresh nutrient agar plates and obtained pure cultures. The pure culture was stored at 4°C for further studies.

2.2.7 Determination of Antibacterial Activity

Agar well diffusion technique was used to determine the antibacterial activity for both crude extract and fractions using Muller Hinton agar medium. The medium was prepared and allowed to solidify, left on the table for about 18-24 hrs for checking the sterility of the medium. The positive control used was Ampicillin 250 mg (standard drug). The distilled water was used as negative control. The crude extract of *Acacia nilotica* leaves were incorporated into five different wells with concentrations of 60, 80 and 100 mg/ml on the inoculated plates. The fractions of *Acacia nilotica* leaves (B1-B5) were incorporated into three different wells with concentrations of 60 µg/ml for each fraction on the inoculated plates. The diameters of zones of inhibition were measured using millimeter ruler (in mm) after incubation for 24 hrs at a temperature of 37 ^{o}C [11].

2.2.8 Statistical Analysis

Quantitative phytochemicals experiment was carried out in triplicates and results are expressed as mean values with standard deviation (± SD) of three replicates. All other experiments were carried out in triplicates and results were expressed as mean values with standard error of mean (± SEM) of the three replicates. Two-way analysis of variance (ANOVA) and Duncan's multiple range tests were carried out to determine significant group differences ($p < 0.05$) between means by using SPSS statistical software package (SPSS, version 21.0).

2.2.9 FT-IR

Exactly 10 mg of plant material was carefully mixed with 100 mg of potassium bromide (KBr), followed by exerting an appropriate pressure to a transparent pellet. The Fourier Transform Infrared (FT-IR) spectra of the plant materials were recorded between the ranges from 4000 to 400 cm^{-1}. The Fourier Transform Infrared (FT-IR) spectra was collected on a Nicolet 6700 Fourier Transform Infrared (FT-IR) (Thermo Scientific, Madison, WI, USA). The Smart ITR ATR sampling accessory was used. The spectra of the plant materials were collected at the range of 4000–650 cm^{-1}. The spectrum of the Fourier Transform Infrared (FT-IR) was used to identify the possible functional groups of the active chemical components that are presents in plants materials, based on the peak standards (values) in the region of Infrared (IR) radiation. When the plants materials (extract) was passed into Fourier Transform Infrared (FT-IR), the possible functional groups of the components were separated according their peaks ratio.

2.2.10 GC-MS

Gas Chromatography Mass Spectrometry (GC-MS) was done by using the GC clarus 500 Perkin Elmer apparatus, biochemical compounds were separated on Elite 5MS (5%) Diphenyl / 95% Dimethyl polysiloxane, 30X0.25mmX0, 25umdf capillary column. The plant materials were inserted with a split ratio of 10:1 that has a flow rate of helium 1m /min as carrier gas. The Turbo mass gold Perkin Elmer Mass detector was used and Turbo mass 5.2 2ul software was used as the sample was injected. Other condition monitored was as oven temperature up to 110^0C – 2min hold, up to 200^0c at the rate of 10^0C/min on hold. The Injector temperature was retained at 250^0C. The chemical constituents were analyzed and identified after comparison with those available in the National Institute of Standard and Technology library (NIST version 2005), attached to the Gas Chromatography Mass Spectrometry (GC-MS) instrument and documented. Analysis of mass spectrum Gas Chromatography Mass Spectrometry (GC-MS) was done using the database of National Institute Standard and Technology (NIST) comprising of more than sixty-two thousand (62,000) patterns, the spectrum of the unknown chemical compound was compared with the spectrum of the known chemical compound that is stored in the National Institute of Standard and Technology (NIST) library. The names, molecular formula, molecular weight, and structures of the chemical compound of the tested plant materials were ascertained.

The biochemical compounds in the fraction of the leaves of *A. nilotica* were analyzed based on Gas Chromatographic (GC) retention time on VF-5 capillary column. The chromatogram was interpreted by using National Institute of Standard and Technology (NIST) library, which consist

about two hundred thousand of biochemical compounds. The name, molecular weight and percentage of unknown biochemical compounds were assessed by the software.

3. RESULTS AND DISCUSSION

3.1 Results

Phytochemical analysis indicated the presence of terpenoids, flavonoids, phenols, glycosides, saponins, alkaloids, steroids and tannins.

Table 1. Results of phytochemical screening of the crude extracts of *A. nilotica* leaves.

Phytochemicals	**Aqueous Extract Result**
Terpenoids	+
Flavonoids	+
Phenols	+
Glycosides	+
Saponins	+
Alkaloids	+
Steroids	+
Tannins	+

Key: *A. nilotica* = *Acacia nilotica*, + = Present

The result of table 2 Showed the antibacterial activity at different concentrations, with *E. coli* having the higher activity for all the tested concentrations, followed by *S. aureus* and *S. typhi* with lowest activity. The positive (Ampicillin) control showed the highest activity compared to the tested concentrations.

Table 2.: Antibacterial activity of the crude aqueous leaves extracts of *Acacia nilotica.*

	Zones of Inhibition (mm)				
Bacterial Isolate	**Distilled H_2O**	**Ampicillin**	***Acacia nilotica* leaves extracts**		
		250 mg/ml	**60 mg/ml**	**80 mg/ml**	**100 mg/ml**
E. coli	0.00±0.00^a	24.10±0.00^c	13.23±0.00^c	17.53±0.03^c	19.23±0.03^c
S. aureus	0.00±0.00^a	23.26±0.03^b	12.53±0.03^b	14.30±0.03^b	17.36±0.03^b
S. typhi	0.00±0.00^a	21.23±0.03^a	11.66±0.03^a	12.53±0.03^a	14.43±0.03^a

Key: *E. coli* = *Escherichia coli, S. aureus* = *Staphylococcus aureus, S. typhi* = *Salmonella typhi* and H_2O = Water. Values are mean inhibition zones (mm) ± SEM of the three replicate experiments. Mean

value having different superscripts letters (abc) along rows are significantly different (P<0.05) while values with the same superscripts letters in rows, are non-significance (P>0.05).

The result of table 3 showed that sub-fraction B3 and B4 shows antibacterial activity, while the sub-fraction B1, B2 and B5 showed zero activity.

Table 3. Antibacterial activity of aqueous column sub-fractions of *Acacia nilotica* leaves.

		Bacteria Isolates/Zone of inhibition (mm)				
S/N	**Column Fractions (60μg/ml)**	***E. coli***	***S. aureus***	***S. typhi***	**Ampicillin 250 mg/ml**	**Distilled Water**
1	B1 (1-11)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	20.13±0.03^{c}	0.00±0.00^{a}
2	B2 (12-29)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	19.53±0.03^{b}	0.00±0.00^{a}
3	B3 (30-46)	7.36±0.03^{c}	4.53±0.03^{b}	1.23±0.03^{b}	22.26±0.03^{e}	0.00±0.00^{a}
4	B4 (47-63)	0.53±0.03^{b}	0.00±0.00^{a}	0.00±0.00^{a}	21.46±0.03^{d}	0.00±0.00^{a}
5	B5 (64-83)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	18.23±0.03^{a}	0.00±0.00^{a}

Key: *E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, S. typhi = Salmonella typhi* and H_2O = Water. Values are mean inhibition zones (mm) ±SEM of the three replicate experiments. Mean value having different superscripts letters (abc) along rows are significantly different (P<0.05) while values with the same superscripts letters in rows, are non-significance (P>0.05).

The FT-IR analysis presented in table 4 revealed the presence of OH, C-H, =C-H, C=O, C=C, C-O functional groups as shown in figure 1.

Table 4. Summary of the FTIR spectrum of sub-fraction B3 of *Acacia nilotica* leaves.

S/NO	**Peak Values (cm^{-1})**	**Possible Functional Groups**	**Intensity of Peak**	**Type of Vibration**	**Possible Compounds**
1	3302.69	O-H	Strong	Stretch, H-bonding	Alcohol/Phenols
2	2937.17	C-H	Strong	Stretch	Alkane
3	2821.68	=C-H	Medium	Stretch	Aldehyde

4	1649.39	C=O	Strong	Stretch	Amide
5	1416.84	C=C	Medium-Weak	Stretch	Aromatic
6	1116.38	C-N	Medium-Weak	Stretch	Amine
7	1019.42	C-O	Strong	Stretch	Ester

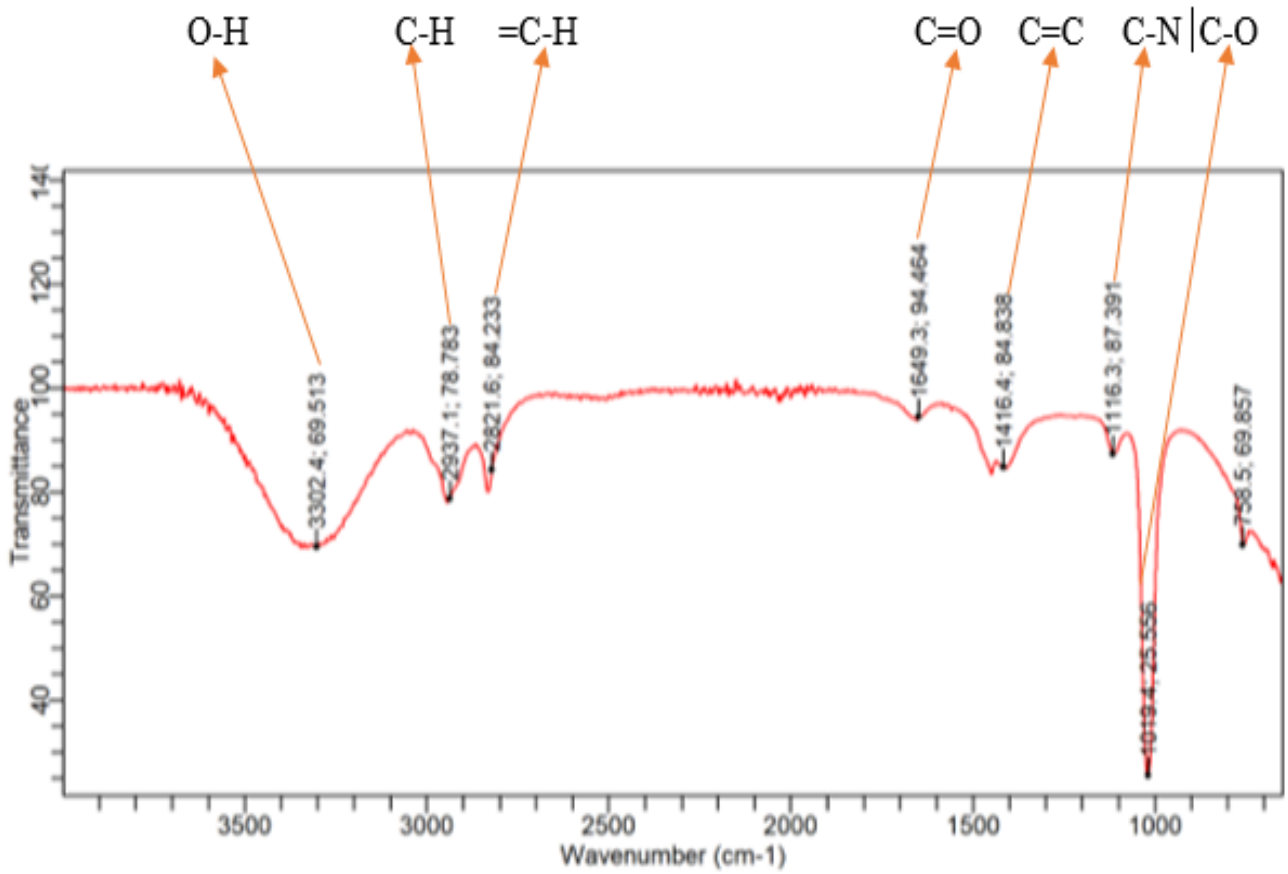

Figure 1. FTIR Spectrum of Sub-fraction B3.

The result of GC-MS analysis presented in Table 5 showed the presence of different biochemical compounds as shown in figure 2, like undecane, tridecane, pentadecane, tridecanal, tetradecane, 4-O-Methylhexopyranose, 1,2-Benzenedicarboxylic acid, hexadecanoic acid and N,N-bis [2-Trimethylsiloxyethyl] ethanamine.

Table 5. Summary of the GC-MS spectrum of sub-fraction B3 of *Acacia nilotica* leaves.

S/NO	RT (min)	% PEAK AREA	METABOLITES	MW
1	11.341	2.42	Undecane	156
2	12.738	4.37	Undecane	156
3	14.048	4.29	Tridecane	184
4	15.276	3.11	Tridecane	184
5	16.458	2.20	Pentadecane	212
6	16.890	1.29	Tridecanal	198
7	17.564	1.51	Tetradecane	198
8	19.358	11.61	4-O-Methylhexopyranose	194
9	20.118	64.35	1,2-Benzenedicarboxylic acid	390
10	21.174	1.27	Hexadecanoic acid	270
11	23.760	3.58	N,N-bis [2-Trimethylsiloxyethyl] ethanamine	277

Key: RT = Retention time (min), % Peak Area = Percentage peak area, MW = Molecular weight.

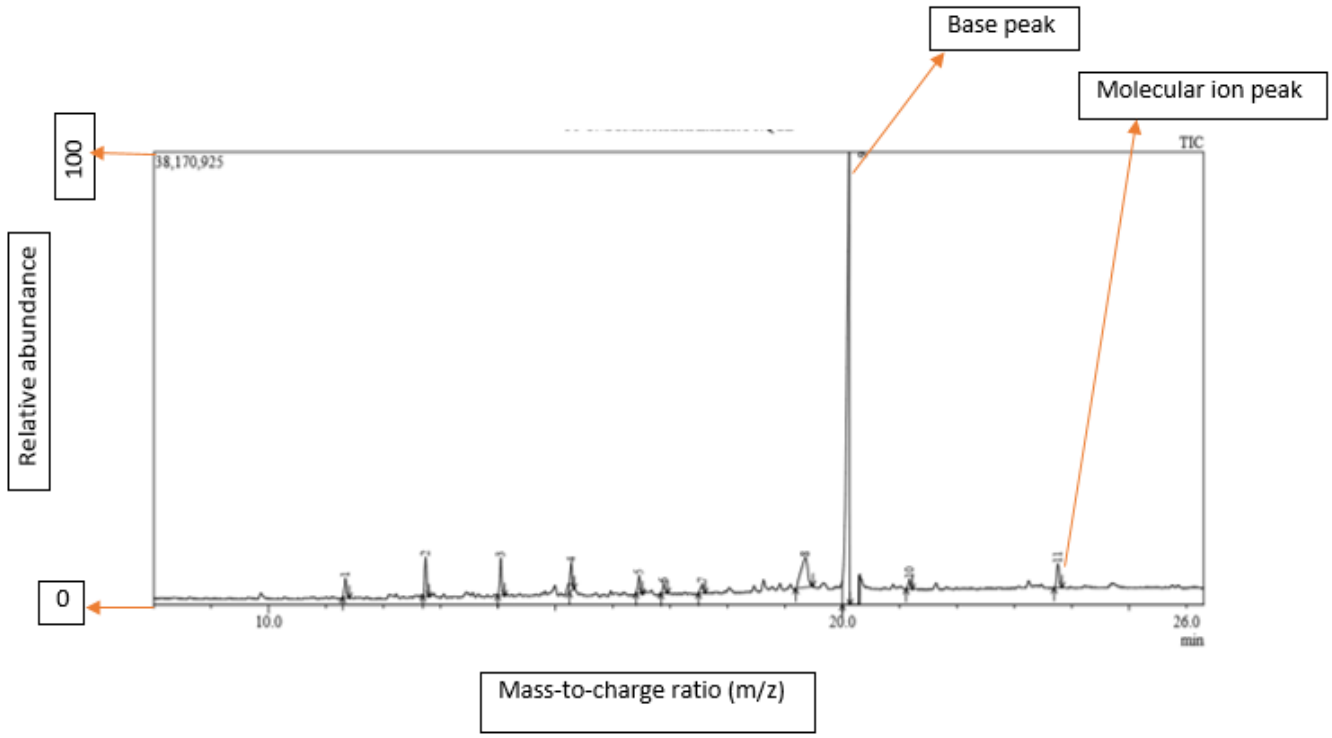

Figure 2. GC-MS Spectrum of Sub-fraction B3.

3.2 Discussion

Medicinal plants play an important role in pharmaceutical industries, as herbal medicines and their derivative products are often prepared from crude plant extracts, which contain a complex mixture of diverse phytochemical components. Bioactive chemical compounds extracted from plants particularly higher plants have been suggested as an alternative source for antibiotics.

The result of table 2 Indicated the antibacterial activity at different concentrations, with *E. coli* having the higher activity for all the tested concentrations (13.23±0.00, 17.53±0.03 and 19.23±0.03 mg/ml), followed by *S. aureus* (12.53±0.03, 14.30±0.03 and 17.36±0.03 mg/ml) and *S. typhi* with lowest activity (11.66±0.03, 12.53±0.03 and 14.43±0.03 mg/ml). The positive (Ampicillin) control showed the highest activity compared to the tested concentrations. The result of table 3 indicated that sub-fraction B3 and B4 shows antibacterial activity, while the sub-fraction B1, B2 and B5 showed zero activity. *E. coli* showed the higher activity at sub-fraction B3 (7.36±0.03 mg/ml) compared to *S. aureus* and *S. typhi* with activity of 4.53±0.03 and 1.23±0.03 respectively. At sub-fraction B4, only *E. coli* showed the activity of 0.53±0.03 mg/ml. The positive control (Ampicillin) showed the high activity for all the tested fractions against bacterial isolates. This study correlates with different researches. The result of antibacterial activity of this study is in agreement with that [12] who confirms the antibacterial activity of *Acacia nilotica* against *E. coli, K. pneumonia, Proteus spp, P. aeruginosa, S. aureus, S. typhi* and *S. pneumonia* using agar well diffusion method. The finding of this study at sub-fraction B1, B2, and B5 also correlate with that of [13] who confirms zero antibacterial activity of *Acacia nilotica* against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Streptococcus pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus vulgaris* and *Salmonella typhi* using the agar disc diffusion method. This study is also related to the findings of [14] against *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* using the agar well method. The result of antibacterial activity of both crude extracts and sub-fractions indicated more active against *E. coli* followed by *S. aureus* and lastly against *S. typhi*. The presence of bioactive chemical compounds in table 5 and presence of phytochemical constituents in table 1 might be responsible for the antibacterial activity in both crude extracts and fractions.

The results of FT-IR in Table 4 indicated a wide stretching of O-H group at peak value (3302.69 cm^{-1}) which indicated the presence of alcohol/phenols with strong intensity and stretching vibration, C-H group at peak value (2937.17 cm^{-1}) indicated the presence of alkane with strong intensity and stretching vibration, =C-H group at peak value (2821.68 cm^{-1}) indicated the presence of aldehyde with medium intensity and stretching vibration, C=O group at peak value (1649.39 cm^{-1}) indicated the presence of carbonyl with strong intensity and stretching vibration, C=C group at peak value (1416.84 cm^{-1}) indicated the presence of aromatic with medium-weak intensity and stretching vibration, C-O group at peak value (1116.38 cm^{-1}) indicated the presence of amine with strong intensity and stretching vibration and C-O group at peak value (1019.42 cm^{-1}) indicated the presence of ether with strong intensity and stretching vibration. The present study is in agreement with the result of [15], which shows numerous vibrations and stretches. The result shows a wide stretch of asymmetric O-H group at peak value (387.03 cm^{-1}), O-H stretching at peak value of (3391.94 cm^{-1}), C-H aliphatic stretching at peak value of (2920.32 cm^{-1}), C=O stretching at peak value of (1635.69 cm^{-1}), C-H plane bending at peak value of (1403.33 cm^{-1}), C-H

wag at peak value of (1285.6 cm^{-1}), C-O stretching at peak value of (10554.13cm^{-1}) and =C-H which is out of plane at peak value of (887.28 cm^{-1}). The result is also similar to the result of [16], which shows 3456 cm^{-1} and 2939 cm^{-1} as high wave region, with asymmetric and symmetric stretching vibrations credited to -OH, -CH and -CH2 functional groups. The detected band at 3273 cm^{-1} indicated the presence of O-H, C=O stretching of –COOH functional group. The remaining spectra were assigned to skeletal bending bonds and bending vibrations of CH, C-O-C and CH3 functional groups. The result is also in agreement with the result of [17], which revealed that, the intermolecular hydrogen bonding among the OH functional groups revealed the evidence of from the broadness of the peak. The peak 1611, 1528 and 1447 cm^{-1} are due to presence of aromatic rings. The peak at 1355 cm^{-1} was due to bending in-plane of the OH functional groups.

The result of GC-MS in Table 5 indicated a total number of 11 bioactive phytochemical Compounds from the column fractions of aqueous leaves extract of *Acacia nilotica.* The identified compounds are Undecane with percentage peak area of 2.42 and molecular weight of 156, Undecane with percentage peak area of 4.37 and molecular weight of 156, Tridecane with percentage peak area of 4.29 and molecular weight of 184, Tridecane with percentage peak area of 3.11 and molecular weight of 184, Pentadecane with percentage peak area of 2.20 and molecular weight of 212, Tridecanal with percentage peak area of 2.20 and molecular weight of 198, Tetradecane with percentage peak area of 1.51 and molecular weight of 198, 4-O-Methylhexopyranose with percentage peak area of 11.61 and molecular weight of 194, 1,2-Benzenedicarboxylic acid with percentage peak area of 64.35 and molecular weight of 390, Hexadecanoic acid with percentage peak area of 1.27 and molecular weight of 270 and N,N-bis [2-Trimethylsiloxyethyl] ethanamine with percentage peak area of 3.58 and molecular weight of 277. The present result is in agreement with the result of [18], which indicated the presence of 19 bioactive chemical compounds. The bioactive chemical compounds are N,N-Dimethylglycine, ethylbenzenethiol, pyrogallol, 1,8,11-Heptadecatriene (Z,Z)-, 4-O-methylmannose, 14,17-Octadecadienoic acid, methyl ester, 9,12-Octadecadienoic acid (Z,Z)-, Methyl oleate, Methyl linoleate, Methyl-9-cis 11-trans-octadecadiene, Methyl stearate, 15-Hyydroxypentadecanoic acid, Gycedyl palmitate, Oxiranyl methyl ester octadecenoic acid, 9-Octadecenamide, Phthalic acid and Ergosta-5,22-diene-3-ol. The present result is also indicated presence of some biochemical compounds which is in agreement with the result of [19], like undecane, pentadecane, tridecanal, 4-O-Methylhexopyranose, 1,2-Benzenedicarboxylic acid and hexadecanoic acid.

4. Conclusion

The result of FT-IR analysis in Table 4 is in agreement with the presence of biochemical compounds in Table 5, which might be due to the presence of phytochemicals like tannins, steroids, saponins, glycosides, phenols, alkaloids, flavonoids and terpenoids in the plant leaves extracts. The present study justified the claim that, the plant contained several biochemical compounds with different pharmacological actions. Hence, there is need to isolate and characterized the exact biochemical compounds that are responsible for antibacterial activity.

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References

[1] I. Raskin, D. M. Ribnicky, S. Komarnytsky, N. Ilic, A. Poulev, N. Borisjuk, A. Brinker, D. A. Moreno, C. Ripoll, N. Yakoby, J. M. O'Neal, T. Cornwell, I. Pastor, and B. Fridlender, Plants and human health in the twenty-first century, Trends in Biotechnology **20**, 522-531 (2002).

[2] M. P. Kahkonen, A. I. Hopia, H. J. Vuorela, J. P. Rauha, K. Pihlaja, T. S. Kujala, and M. Heinonen, Antioxidant activity of plant extracts containing phenolic compounds, Journal of Agricultural and Food Chemistry. **47**, 3954-3962 1999,

[3] E. Karimi, and H. Z. E. Jaafar, GC-MS determination of bioactive compounds in microwave obtained extracts of three varieties of Labisia pumila benth, Molecules **16**: 6791– 6805 (2011).

[4] P. Vuorela, M. Leinonen, P. Saikku, P. Tammela, J. P. Rauha, T. Wennberg, H. Vuorela, Natural products in the process of finding new drug candidates, Current medicinal chemistry **11**(11):1375-89 (2004).

[5] B. Obadomi, and P. Ochuko, Phytochemical studies comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria, Global Journal Pure Applied Sciences **8**: 203-208 (2001).

[6] O. H. Edeoga, D. E. Okwu, and B. O. Mbaebie, Phytochemical constituents of some Nigerian medicinal plants, African Journal of Biotechnology **4**: 685–688 (2005).

[7] P. Parmar, S. Bhatt, S. Dhyani, and A. Jain, Phytochemical studies of the secondary metabolites of Ziziphus Mauritiana, International Journal of Current Pharmaceutical Research **4**(3), 3-5 (2012).

[8] D. R. Davies, and T. M. Johnson, Isolation of Three Components from Spearmint Oil: An Exercise in Column and Thin-Layer Chromatography, Journal of Chemistry Eductaion **84**(2): 318 (2007).

[9] M. S. Jonathan, N. Lien, M. Hector, and N. Kelly, TLC plates as a convenient platform for solvent-free reactions, Chemical Communications **12**, 1240 – 1241 (2007).

[10] S. Rajvaidhya, G. K. Singh, B. P. Nagori, and S. Alok, Extraction, isolation and chemical structure elucidation of daidzein from bark of Acacia arabica (Lam.) Willd of Bhopal, Madhya Pradesh, India, International Journal of Pharmaceutical Sciences and Research *5*(5) (2014).

[11] N. O. Klastrup, Scandinavian recommendation on examination of quarter milk samples. Proceedings of International Dairy Federation annual bulletin **85**, 49-52 (1975).

[12] A. Abdulhamid, Y. U. Dabai, and A. M. Ismail, Preliminary phytochemical screening and Antibacterial properties of crude leaves extract and fractions of Acacia nilotica (Linn.), World Journal of Pharmaceutical Research *7*(5), 8-18 (2018).

[13] S. O. Okoro, A. H. Kawo, and A. H. Arzai, Phytochemical screening, antibacterial and toxicological activities of *Acacia nilotica* extracts, Bayero Journal of Pure and Applied Sciences **7**(1), 105-115 (2014).

[14] Z. I. Khaleel, F. Aminu, L. Mu'azu, and M. Ali, Antifungal activity and phytochemical screening of *Acacia nilotica* leaf extracts against isolates of some bacteria, Journal of Biopolymers and theoretical studies **1**(1), 01-06 (2021).

[15] O. A. Paul, Synthesized iron oxide nanoparticles from *Acacia nilotica* leaves for the sequestration of some heavy metal ions in aqueous solutions, Journal of Chemistry Letters, **4**, 38-51 (2023).

[16] F. M. Luvincia, B. R. Abhishek, and P. Vivek, Phytochemical, FTIR and NMR analysis of crude extract of *Acacia planifrons* seeds, Journal of Pharm. Sci. & Res. **11**(5), 1960-1962 (2019).

[17] R. Majumdar, B. G. Bag, and N. Maita, *Acacia nilotica* (Babool) leaf extract mediated size-controlled rapid synthesis of gold nanoparticles and study of its catalytic activity, International nano letters **3**(53), 1-6 (2013).

[18] N. U. Rehman, M. N. Ansari, W. Ahmad, and M. Amir, GC–MS analysis and in vivo and ex vivo antidiarrheal and antispasmodic effects of the methanolic extract of *Acacia nilotica. Molecules*, **27**(7), 2107 (2022).

[19] S. Bai, L. Seasotiya, A. Malik, P. Bharti, and S. Dalal, GC-MS analysis of chloroform extract of Acacia nilotica L. leaves, Journal of Pharmacognosy and Phytochemistry **2**(6), 79-82 (2014).