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Research Article

ISOLATION AND CHARACTERIZATION OF COMPOUND FROM THE LEAF EXTRACT OF ALBIZIA CHEVALIERI

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ABSTRACT

Background:
Albizia chevalieri is a medicinal plant that grows up to 12 m height under temperate condition of the dry savanna in the republic of Niger, Nigeria, Senegal and Cameroon. This study was performed to isolate and characterize a compound from the leaf extract of Albizia chevalieri.
Methods: The crude extract was obtained by macerating the powdered sample of Albizia chevalieri which was further fractionated with n-hexane, dichloromethane, acetone and methanol solvents. The dichloromethane was subjected to column chromatography packed with silica gel for the isolation of compound. The isolated compounds were further purified using Preparative Thin Layer Chromatography which yielded compound. The isolated compound was characterized by subjecting it to Fourier Transform Infrared Resonance Spectroscopy (FTIR), and Proton and Carbon-13 Nuclear Magnetic resonance spectroscopy (proton and carbon-13 NMR) techniques
Results: From the results, it has been observed that acetone extract has the highest percentage yield (50.15 %), followed by n-hexane extract (25 %), dichloromethane extract (16.87 %), ethanol extract (12.00 %), and then methanol extract (7.70 %), respectively. FTIR analysis reveals the presence of -OH, -C-H, C=O, and C-O. functional groups. Chromatographic separation revealed a compound obtained at a retention factor of 0.4. The compound isolated was named 3a-Methyl-oxo-1-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-octahydro-indene-5-carboxylic acid using the information provided by the FTIR and NMR, respectively.
Conclusion: The result of this research could pave the way for the evaluation of the pharmacological activities of the isolated compound, thereby providing scientific validation for the ethnomedicinal usage of Albizia chevaieri leaf in the treatment of different numerous diseases.

Keywords: Plant extract, *Albizia chevalieri, Extraction, Chromatography, Medicinal Plant*

INTRODUCTION

The study of traditional medical practices that focuses on the cultural or traditional conceptions of health, illness, and disease is known as ethnomedicine [1]. People have been using ethnomedicine, a multidisciplinary system that uses plants, animals, and the natural environment to heal for thousands of years. This makes ethnomedicinal studies vital not only to the next generation but also for pharmaceutical firms for the creation of new medications [2, 3].

A significant component of traditional medicine, which is ingrained in African culture, is the utilization of medicinal plants [4]. The evolution of human civilization has been significantly influenced by medicinal plants. In almost every culture and civilization, medicinal plants have been used as a source of medicine.

Many modern medications are made from medicinal plants, which are considered to be abundant sources of traditional remedies [5]. Medicinal herbs have been utilized for thousands of years to treat illnesses, preserve food, enhance flavor, and stop disease outbreaks. The biological traits of plant species used worldwide are typically caused by the secondary metabolites that the plants produce. Plant-derived compounds regulate microbial development in a variety of settings [6].

According to recent WHO figures, infectious diseases have killed over 14 million people globally, with many people at high risk. Additionally, it was stated that the second greatest cause of death worldwide was bacterial infections [7]. These alarming epidemiological findings have been ascribed to bacterial resistance to antibiotics, which has also been found to be the direct cause of roughly 2 million deaths and the indirect cause of 5 million deaths [8]. By 2050, it is predicted that this will cause 10 million deaths annually, more than malignant malignancies. Unfortunately, the low- to middle-income economies have remained the hardest hit by this dilemma due to poor hygiene, poverty, abuse and overuse of antibiotics and lack of accessibility to competent healthcare [9]. For their medical needs, most individuals in low-income nations turn to complementary and alternative therapies. In addition to African and European traditional remedies, this has resulted in the development of homoeopathy, naturopathy, Siddha, Unani, and yoga [10]. There is conjecture that the prevalence of certain plant species used to treat endemic diseases in a given area is closely linked to those diseases' endemicity [11].

Albizia chevalieri is a tree that can reach a height of 12 meters and can also grow as a shrub in the arid savannahs of Senegal, Niger, and Nigeria. Its canopy is open, rounded, or umbrella-shaped, its bark is pale-grey, its twigs are pubescent with white lenticels, and its leaves have 20–40 pairs of leaflets and 8–12 pairs of pinnates each. The bark was found to contain alkaloids and also tannins sufficient for use in tanning in Nigeria and Senegal. In Borno, northeastern Nigeria, it is used as a cough remedy, taenicide, and purgative. In Northern Nigeria, a leaf decoction is used to treat dysentery [12].

The leaf extract of Albizia chevalieri is used as an alternative medication in traditional medicine to treat diabetes mellitus. By extending the infected Rat's survival period, Albizia chevalieri demonstrates dose-dependent efficacy against the Plasmodium parasite [13].

MATERIAL AND METHODS

Apparatus and Reagents/Chemicals

The following tools were used in this study: beakers, a measuring cylinder, a conical flask, a round bottom flask, a flat bottom flask, a glass rod, a glass funnel, a glass column, a dropper, a capillary tube, a Pasteur pipette, cotton wool, and a preparative TLC glass plate. During the investigation, the following chemicals and reagents were used: n-hexane, dichloromethane, acetone, methanol, distilled water, iodine crystal, silica gel (60-120 mesh), and a pre-coated TLC plate measuring 20 × 20 cm. All the chemicals used are of laboratory standard [14].

Sample Collection and Identification

The fresh leaves of Albizia chevaleiri were collected from Gangaran Dutse village in Dawakin Tofa Local Government Area of Kano State. The leaves of the plant were identified and authenticated by Dr. Yusuf Nuhu at the Herbarium unit of the Department of Plant Biology, Bayero University, Kano, Nigeria with an accession number BUKHAN 378 [14].

Sample Preparation

The collected fresh leaves of Albizia chevalieri were washed with distilled water to remove dirt and other foreign contaminant. The washed leaves were air-dried under shade for several days and crushed into coarse powder using wooden mortar and pestle. The powdered samples were weighed (500 g) and stored in an air tight container [14].

Extraction and Fractionation of the powdered plant materials

The powdered leaves of Albizia chevalieri (500 g) was extracted using ethanol (1.5 L) for one week and filtered using Whatmann filter paper. The filtrate was concentrated using rotary evaporator at temperature 43 0C under reduced pressure and dried under shade to obtain a dark green crude dry extract. The ethanol crude of Albizia chevalieri leaf (60.0 g) was fractionated successively using n-hexane, dichloromethane, acetone and methanol in the order of increasing solvent polarity [14].

Column Chromatography

The dichloromethane fraction (10.0 g) was dissolved in a small portion of dichloromethane solvent. Thereafter, 10.0 g of silica gel was added and stirred thoroughly with a glass rod in 100 ml beaker. The mixture was kept under shade to dry. The dried mixture was subjected to column chromatography on 300 g silica gel (60-120 mesh). Dichloromethane and ethyl acetate solvents were used in the elution process as solvents system in the ration of 100:0, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95, and 0:100. A total of 69 fractions were collected and preserved for further analysis [15].

Preparative Thin Layer Chromatography

The semi purified compounds obtained from column chromatographic separation of the dichloromethane fractions of Albizia chevalieri leaf were combined together according to their retention factors and the sub-fractions were purified by preparative TLC performed on silica gel pre-coated plate (10 x 20 cm) dimension, thickness 0.5cm and developed with mobile system (hexane:

dichloromethane, 2:3), the separated pure compounds were viewed under iodine crystal. Bands at Rf = 0.4 were scraped off and dissolved in acetone. The solvent was allowed to dry under shade to yield compound ACF2 [16].

FTIR and NMR Analyses

The isolated compound ACF2 from dichloromethane fraction was subjected to FTIR, NMR, and MS techniques for identification. The FTIR was analyzed on spectrophotometer operating at 1000 − 4000 cm-1 with KBr pellet that confirmed peaks at different wave number ranges. For the NMR (1HNMR and 13C NMR), the isolated compound was dissolved in acetone and analyzed on BRUKER at a temperature of 300 K and frequency of 300.00 MHz [17].
Characterization of the Isolated Compound

RESULTS

Percentage yields of the extracts

The table below contains the color, texture, weights, and yields of the extracts from the leaf of Albizia chevalieri.

Table 1: Physical properties of the extracts of Albizia chevalieri.

Extracts	Color	Texture	Weight (g)	Yield (%)
Ethanol	Dark Green	Sticky Solid	60	12.00
N-Hexane	Dark Brown	Oily	15.17	25.28
Dichloromethane	Greenish	Solid	10.12	16.87
Acetone	Greenish Brown	Solid Crystals	30.09	50.15
Methanol	Reddish Brown	Solid Crystals	4.62	7.70

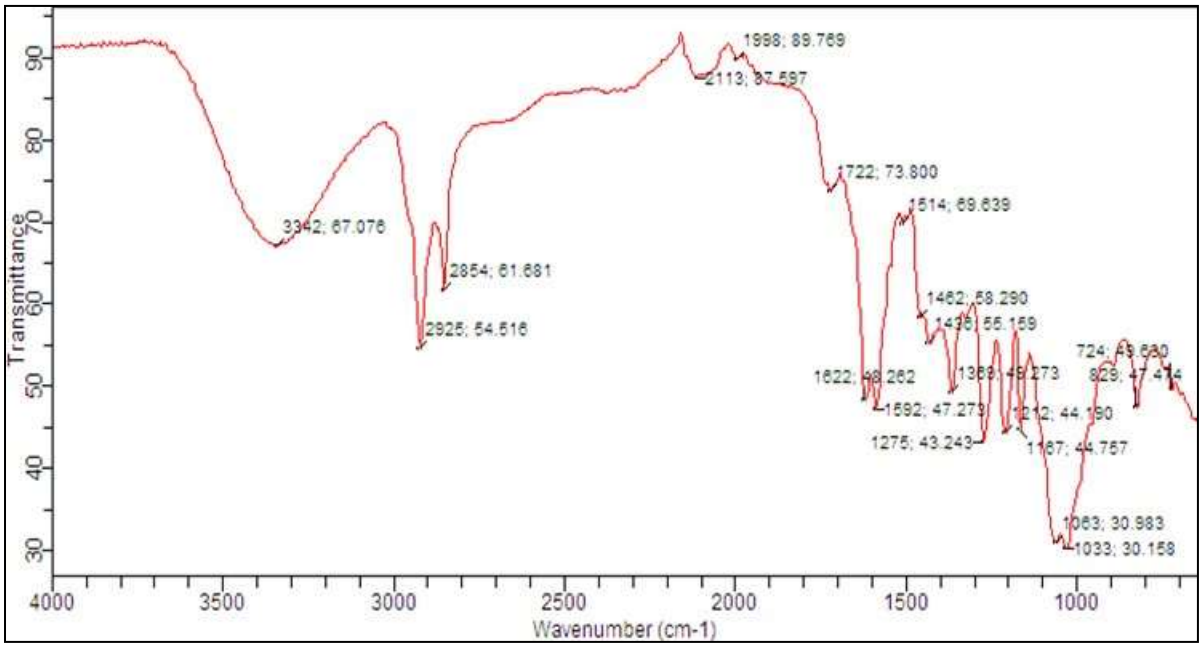

Fig 1: FTIR spectra of the isolated compound from Albizia chevalieri

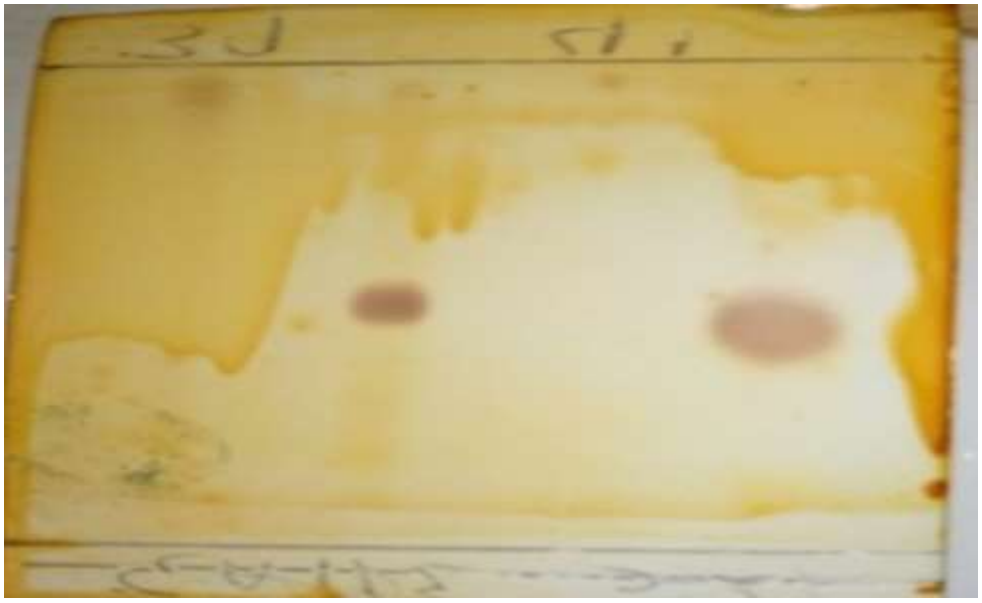

Fig 2: Spots of the isolated compound on the TLC plate

Table 2: Proton NMR spectra of the isolated ACF2

Chemical shift (ppm)	Types of Protons	Number of protons
0.874	-CH3	3
2.084	CH-OH	4
3.857	- CH2-OH	1
13.845	COOH	1
3.674	O-CH	2
1.297	CH2-C	4
1.608	CH-C	2

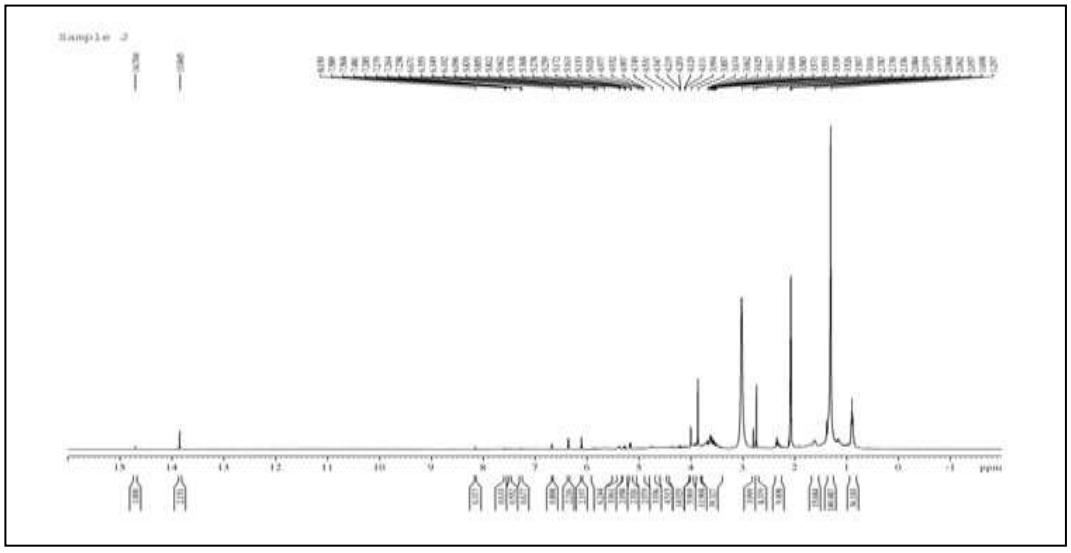

Fig 3: 1H NMR Spectra of the isolated compound

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Table 3: 13-C NMR chemical shifts of the isolated compound

^{13}C Position	Chemical Shifts (ppm
C1	101.38
C2	95.219
C3	77.620
C4	73.575
C5	93.490
C6	70.269
C7	56.347
C8	63.478
C9	55.170
C10	22.419
C11	24.739
C12	33.560
C13	166.700
C14	31.728
C15	61.873
C16	13.445
C17	203.691

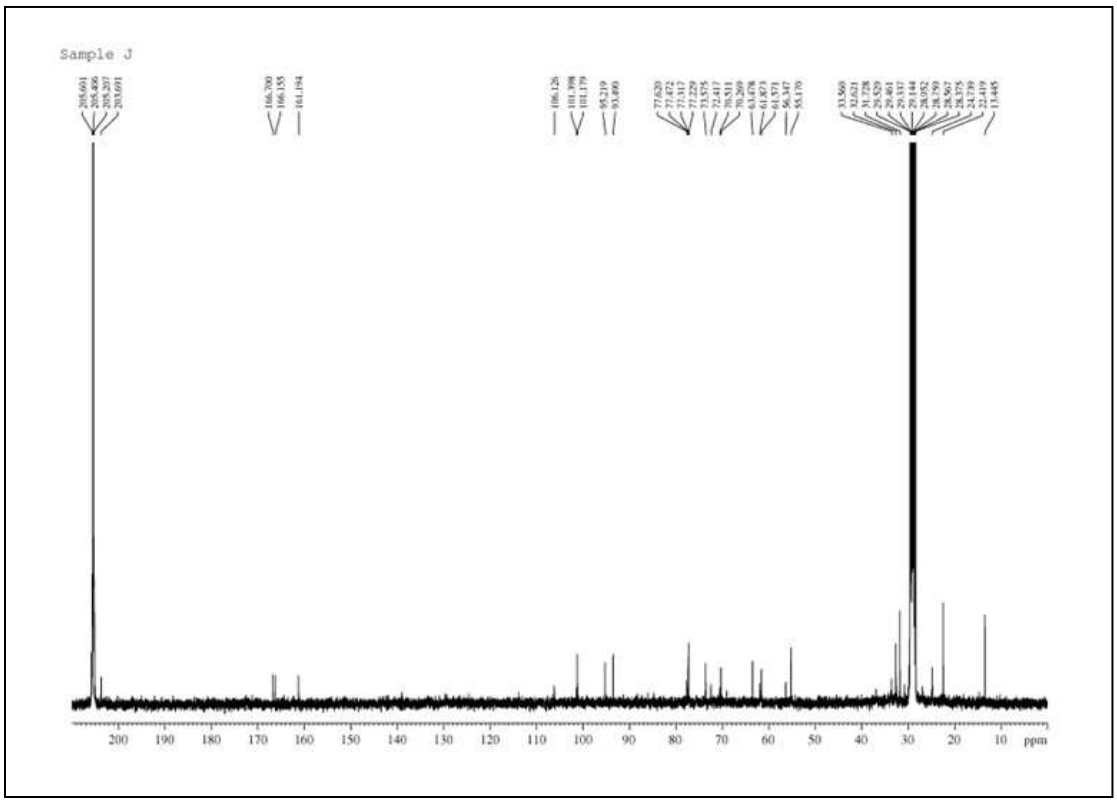

Fig 4: 13C NMR spectra of the isolated compound

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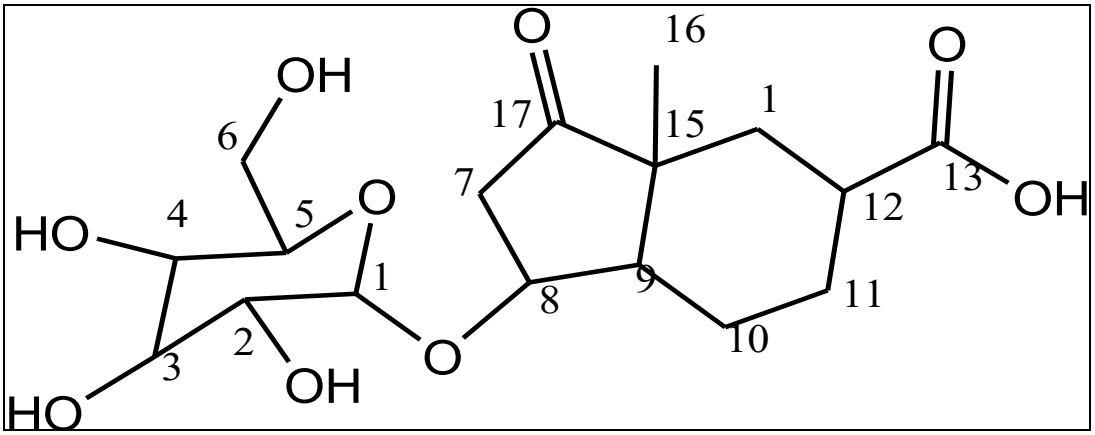

Fig 5: Proposed isolated compound from the leaf extract of Albizia chevalieri: 3a-Methyl-oxo-1-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-octahydro-indene-5-carboxylic acid

DISCUSSION

Table 1 shows the physical properties of the extracts in this study. From the results, it has been observed that acetone extract has the highest percentage yield (50.15 %), followed by n-hexane extract (25 %), dichloromethane extract (16.87 %), ethanol extract (12.00 %), and then methanol extract (7.70 %), respectively.

The Fourier transform infrared (FTIR) spectra (figure 1) of the isolated compound from dichloromethane fraction (ACF2) was presented revealed absorption bands at 3346 cm-1 which is attributed to the presence of hydroxyl functional group (–OH). Absorption bands at 2925cm-1 and 2854cm-1 were due to the presence of carbon-hydrogen stretching (C-H) from aliphatic asymmetric compound; a band at 1722cm-1 indicate the presence of carbonyl group (C=O), and an ether carbon-oxygen single bond (C-O) was seen as an absorption band at 1033 cm-1 respectively.

The Proton NMR (1H NMR) of the isolated compound in this study (table 2 and figure 3) shows signals indicating the presence of -CH3 at 0.874 ppm, three hydroxyl groups at 2.084 ppm, 3.857 ppm, and 13.845 pm. The -OH at a signal 13.845 was the -OH attached to the carbonyl carbon. There is also a signal showing -CH at 3.674 ppm, another -CH at 1.608 ppm, and a -CH2 at 1.297 ppm, respectively.

The 13C NMR spectra of Isolated compound (ACF2) was shown in (figure 4) which revealed the presence of seventy (17) major peaks which correspond to the different types of carbon atoms in the compound. The chemical shift values in (ppm) of the carbon peaks are represented in (Table 3). From the information provided by the FTIR and NMR, a compound "3a-Methyl-oxo-1-(3,4,5-trihydroxy-6 hydroxymethyl-tetrahydro-pyran-2-yloxy)-octahydro-indene-5-carboxylic acid" was proposed.

CONCLUSION

Medicinal plants are reservoir of a wide range of chemical compounds which have been playing an important role in traditional medicine in the management of variety of diseases. In this study, 3a-Methyl-oxo-1-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-octahydro-indene-5-carboxylic acid has been isolated and characterized from the dichloromethane fraction of Albizia chevalieri leaf using

chromatographic separation, 1HNMR, 13CNMR, and FTIR spectrometric analysis. However, further research is needed to verify the effectiveness and safety of the isolated compound which may serve as a potential candidate in drug formulation.

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