

## Research Article

### Isolation, antibacterial and antifungal evaluation of $\alpha$ -amyrenol from the root extract of *Acacia ataxacantha* DC

Aba, O. Y<sup>1,2\*</sup>, Ezuruike, I.T<sup>1</sup>, Ayo, R.G<sup>1</sup>, Habila, J. D<sup>1</sup> and Ndukwe, G. I<sup>1</sup>

<sup>1</sup>Department of Chemistry Ahmadu Bello University Zaria-Nigeria

<sup>2</sup>Department of Chemistry Kaduna State University Kaduna-Nigeria.

#### \*Corresponding author

Aba, O. Y

Email: [opheliayaba@gmail.com](mailto:opheliayaba@gmail.com)

**Abstract:** The root-bark of *Acacia ataxacantha* (Fabaceae) was investigated for medicinal potential. The Phytochemical screening indicated presence of flavonoids, glycosides, saponins, steroids/triterpenes, tannins and alkaloids. The crude extracts and  $\alpha$ -amyrenol were evaluated for antibacterial and antifungal potentials, using the following clinical isolates; *Bacillus subtilis*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans* and *Candida krusei*. The zone of inhibition of the test organism ranges from 17 – 19 mm (Pet. ether), 20- 25 mm (chloroform), 20 – 30 mm (ethyl acetate), 20 – 23 mm (methanol) and 25 – 31 mm ( $\alpha$ -amyrenol). These compared favourably with Sparfloxacin (35 – 42 mm) and Cefuroxime (34 – 40 mm) used as positive control. No activities were observed on *Corynebacterium ulcerans*, *Streptococcus faecalis*, *Proteus mirabilis* and *Candida tropicalis*. The minimum inhibitory concentration (MIC) of the crude extracts showed that Methanol and chloroform fractions had 5 mg/mL and petroleum ether had 10 mg/mL on the test organisms. Ethyl acetate fraction was the most active, with MIC of 2.5 mg/mL against *B. subtilis*, *E. coli*, *S. Typhi* and *K. pneumonia*. The minimum bactericidal/fungicidal concentration (MBC/MFC) showed that ethyl acetate extract completely kill *B. subtilis*, *E. coli* and *K. pneumoniae* at a concentration of 5.0 mg/mL. Chromatographic purification of the Ethyl acetate extract led to the isolation of  $\alpha$ -amyrenol (3 $\beta$ )-Urs-12-en-3-ol). The MIC and MBC/MFC of  $\alpha$ -amyrenol was found to be 12.5 and 25  $\mu$ g/mL respectively against *B. subtilis*, *E. coli* and *S. typhi*. These seem to justify the numerous folkloric medicinal uses of the plant.

**Keywords:** antimicrobial screening, *Acacia ataxacantha*, chromatography,  $\alpha$ -amyrenol, spectroscopy.

## INTRODUCTION

The use of the medicinal herbs for curing diseases has been documented in the history of all civilizations. The drugs were used in crude forms, like expressed juice, powder, decoction or infusion [1]. The World Health Organization defined medicinal herbs as finished, labelled medicinal products that contain as active ingredients aerial or underground parts of plants, or other plant material, or combinations thereof, whether in the crude state or as plant preparations. The widespread use of plants in folk medicine by traditional medicinal practitioners has continued to create awareness in the study of plants and medicinal plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. The world's tropical rain forests are especially rich in biodiversity but there is rapid depletion of these natural resources worldwide, and in Nigeria in particular, the pressures from degradation, unsustainable arable land use, urbanization and

industrialization are taking their toll as well [2]. Medicinal plants and herbs contain substances known to modern and ancient civilization for their healing properties. Until the development of organic compounds in the nineteenth century, medicinal plants and herbs were the sole source of active principles capable of curing man's ailments. Plants have continued to be major sources of medicine either in the form of traditional medicine preparations or as pure active principles. This has made it important to identify plants with useful therapeutic action for possible isolation and characterization of their active constituents [3].

*Acacia ataxacantha* commonly known as flame thorn belong to the sub-family Mimosoideae of the Fabaceae family [4]. It is a shrubby scrambler, stems grows up to 10 m long forming thicket 4-5 m deep. Distributed around rocky hills in the forest region and in dry savannah areas from Sengal to Nigeria and widespread in tropical Africa. Its choice as the plant of interest in this work was based on its economic relevance and also vast medicinal importance among

traditional medicine practitioners in the tropical regions of Africa. *A. ataxacantha* is extensively used in herbal remedies in the treatment of pneumonia [6, 7], chickenpox [8], excessive cough and yellow fever [9], dysentery and back-ache [10], syphilis and fumigations for maladies of the respiratory tract [5]. Though the chemical studies of some *Acacia* species have been explored to some extent, nothing was reported on *Acacia ataxacantha* to the best of our knowledge. Hence our interest in this plant as possible source of antibacterial compound(s).

## MATERIALS AND METHOD

### Plant material

The plant parts of *Acacia ataxacantha* were collected from Edumoga in Okpokwu Local Government Area of Benue state, Nigeria in March, 2013. The plant was properly identified by the curator of the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The voucher specimen number 1707 was deposited in the Herbarium.

### Extraction

The root-bark was air-dried and pulverized using wooden mortar and pestle. The pulverised sample was subjected to cold extraction using methanol. The crude methanol extract was suspended in water and washed with petroleum ether, chloroform and ethyl acetate in that order to yield petroleum ether fraction (PEF), chloroform fraction (CF) and ethyl acetate fraction (EAF), respectively.

### Phytochemical analysis

The various extracts were subjected to phytochemical screening using standard techniques of plant secondary metabolites [11], [12]. The metabolites tested for were alkaloids, saponins, glycosides, tannins, anthraquinones, cardiac glycosides, steroids/triterpenes and flavonoids.

### Antimicrobial Screening

The sensitivity test was carried out using the paper disc agar diffusion techniques as described by Bauer *et al.*, [13] and Barry and Thornberry [14].

The antimicrobial activity of *Acacia ataxacantha* plant extracts was determined using some pathogenic microorganisms [*Bacillus subtilis*, *Corynebacterium ulcerans*, *Streptococcus pneumonia*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, *Candida tropicalis* and *Candida krusei*] obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria. All the isolates were checked for purity and maintained in slants of nutrient agar.

Well diffusion method [15] was used to check the antimicrobial activities of the plant extracts. 0.2g each of the extracts were weighed and dissolved in 10mL of DMSO to obtain a concentration of 20mg/mL. This was the initial concentration of the extracts used to check the antimicrobial activities of the plant.

### Minimum Inhibitory Concentration (MIC)

The Broth Dilution method [16] was employed in this determination. Mueller Hinton broth was prepared, 10mL was dispensed into test tubes and sterilized at 121°C for 15mins, and the broth was allowed to cool. Mc-Farlands turbidity scale number 0.5 was prepared to give turbid solution. 10mL of normal saline was dispensed into sterile test tubes and the test microorganisms were inoculated into the different test tubes containing the normal saline. Incubation of the normal saline was done at 37°C for 6hrs. Dilution of the test microorganisms in the normal saline was done continuously until the turbidity reached that of the Mc-Farland's scale by visual comparison. At this point the test microbe has a concentration of about  $1.5 \times 10^8$  cfu/mL. Two-fold serial dilution of the extracts in the sterile broth was made to obtain the concentrations of 20mg/mL, 10mg/mL, 5mg/mL, 2.5mg/mL and 1.25mg/mL. The initial concentration was obtained by dissolving 0.2g of the extracts in 10ml of the sterile broth. Having obtained the different concentrations of the extracts in the broth, 0.1mL of the test microorganism in the normal saline was then inoculated into the different concentrations; incubation was done at 37°C for 24hrs after which the broth was observed for turbidity (growth).

### Minimum Bactericidal and Fungicidal Concentration (MBC/MFC)

Mueller Hinton agar was prepared, sterilized at 121°C for 15min, 20ml was poured into sterile petri dishes and was allowed to cool and solidify. The contents of the MIC in the serial dilution were sub-cultured into the prepared medium. Incubation was done at 37°C for 24hrs, after which the plates were observed for colony growth.

### Isolation and purification of compound

A small quantity of EAF was dissolved in ethyl acetate and the solution was spotted on commercially available pre-coated TLC plates (20 × 20 cm). The plates were developed using several solvent systems; the solvent systems of chloroform /ethyl acetate (8:2 and 7:3) gave better separation of the components, and were used in the TLC monitoring of the column chromatography. 6.82 g of EAF was subjected to column chromatography on a silica gel (60 – 120 mesh) and ran first with 100% n-hexane. The polarity of the solvent was increased by mixing with ethyl acetate and chloroform at different ratios and eluents were collected in 20 ml aliquots and TLC was used to monitor the fractions. A total of 108 collections were made and pooled into 9 major fractions based on

their TLC profiles. Fraction 5 indicated significant proportion of the compound of interest and was further subjected to purification by preparative TLC using a solvent system of chloroform /ethylacetate (8:2). A single homogenous spot was obtained on TLC. This compound, labelled ABA, appeared as white crystalline with  $R_f$  value of 0.588 and was subjected to spectral analysis for structural elucidation.

### Spectroscopic Characterization

Different spectroscopic methods were used to elucidate the structure of ABA, including FTIR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and 2D NMR techniques. The FTIR spectrum was recorded on Perkin Elmer Spectrum 8400S (Shimadzu) FTIR Spectrometer at NARICT, Zaria; while the NMR spectra were recorded on a Bruker – Avance (100MHz and 400MHz) in deuterated chloroform at the University of Kwazulu Natal, Westville Campus, Durban.

### Antimicrobial Screening of ABA

The antimicrobial activity of ABA was determined using same pathogenic microorganisms used on the crude extracts. This was done to determine the medicinal properties of the isolated compound ABA. Zones of Inhibition, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal and Fungicidal Concentration (MBC/MFC) were determined using the same procedure as that of the crude extracts except for the concentrations. 0.001 mg each of the compound was weighed and dissolved in 10 mls of DMSO to obtain a concentration of 100  $\mu\text{g}/\text{mL}$ . This was the initial concentration used to check the antimicrobial activities of the isolated compound. Two-fold serial dilution of the extracts in the sterile broth was made to obtain the concentrations of 100, 50, 25, 12.5 and 6.25  $\mu\text{g}/\text{mL}$ . The initial concentration was obtained by dissolving 0.001 mg of the compound in 10 ml of the sterile broth.

## RESULTS AND DISCUSSION

**Table 1: Phytochemical Screening**

Metabolites	ME	EAF	CF	PEF
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Anthraquinones	-	-	-	-
Saponins	+	+	+	+
Tannins	+	+	+	+
Carbohydrates	+	+	+	-
Alkaloids	+	+	+	+
Steroids/ Triterpenes	+	+	+	+

**Key:** - = Absent, + = Present

PEF= petroleum ether fraction, CF= chloroform fraction, EAF= ethyl acetate fraction ME=methanol extract

**Table 2: Diameter of Zones of Inhibition (mm)**

Test organisms	ME	EAF	CF	PEF	SF	CX	FZ	ABA	SF	CX	FZ
<i>S. aureus</i>	20	25	22	18	36	32	0	28	37	38	0
<i>S. pyogenes</i>	21	25	21	17	30	30	0	26	35	34	0
<i>S. faecalis</i>	0	0	0	0	32	29	0	0	36	37	0
<i>S. pneumoniae</i>	23	26	22	18	35	32	0	24	37	35	0
<i>C. ulcerans</i>	0	0	0	0	32	0	0	0	35	0	0
<i>B. subtilis</i>	23	29	25	19	45	40	0	31	42	40	0
<i>E. coli</i>	24	30	25	18	35	30	0	28	35	37	0
<i>S. typhi</i>	20	27	22	18	30	0	0	29	35	37	0
<i>S. enteritidis</i>	20	25	20	17	32	30	0	25	37	0	0
<i>P. mirabilis</i>	0	0	0	0	27	0	0	0	0	35	0
<i>P. aeruginosa</i>	21	24	21	17	0	32	0	27	0	34	0
<i>K. pneumoniae</i>	22	28	24	19	47	40	0	25	37	39	32
<i>C. albicans</i>	20	23	20	18	0	0	32	25	0	0	0
<i>C. tropicalis</i>	0	0	0	0	0	0	29	0	0	0	37
<i>C. krusei</i>	20	20	21	18	0	0	34	24	0	0	35

**Key:** SF= Sparfloxacin; CX= Cefuroxime; FZ = Fluconazole. Their concentrations were 5  $\mu\text{g}/\text{mL}$ .

**Table 3: MIC (mg/mL)**

Test organisms	ME	EAF	CF	PEF	ABA( $\mu\text{g/mL}$ )
<i>S. aureus</i>	5.00	5.00	5.00	10.00	12.50
<i>S. pyogenes</i>	5.00	5.00	5.00	10.00	25.00
<i>S. pneumoniae</i>	5.00	5.00	5.00	10.00	25.00
<i>B. subtilis</i>	5.00	2.50	5.00	10.00	12.50
<i>E. coli</i>	5.00	2.50	5.00	10.00	12.50
<i>S. typhi</i>	5.00	2.50	5.00	10.00	12.50
<i>S. enteritidis</i>	5.00	5.00	5.00	10.00	25.00
<i>P. aeruginosa</i>	5.00	5.00	5.00	10.00	12.50
<i>K. pneumoniae</i>	5.00	2.50	5.00	10.00	25.00
<i>C. albicans</i>	5.00	5.00	5.00	10.00	25.00
<i>C. krusei</i>	5.00	5.00	5.00	10.00	25.00

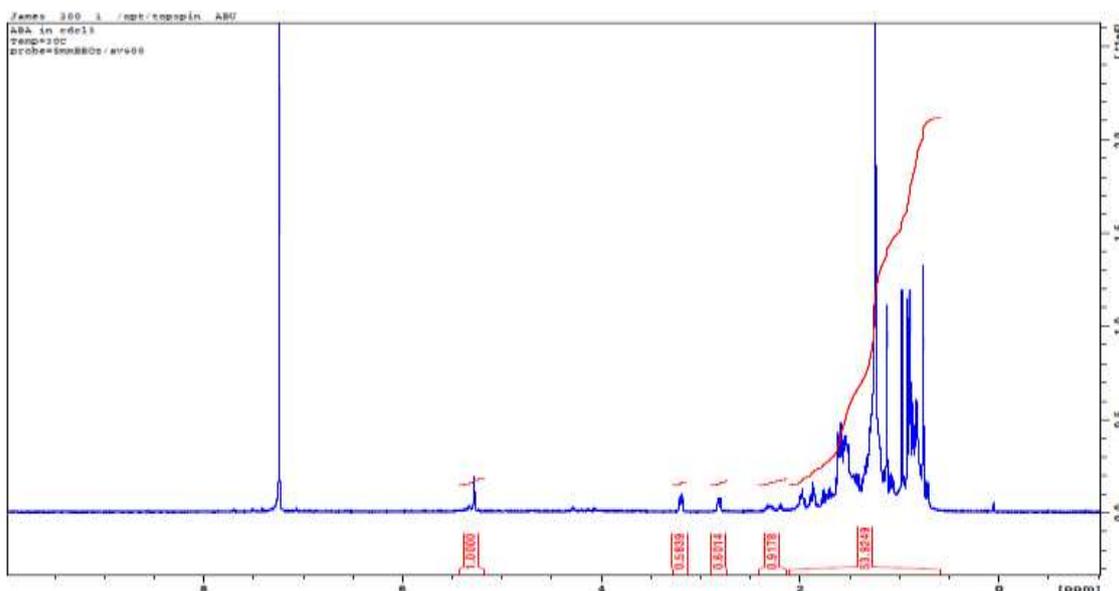
**Table 4: MBC/MFC (mg/mL)**

Test organisms	ME	EAF	CF	PEF	ABA( $\mu\text{g/mL}$ )
<i>S. aureus</i>	20.00	10.00	10.00	20.00	50.00
<i>S. pyogenes</i>	20.00	10.00	20.00	20.00	50.00
<i>S. pneumonia</i>	10.00	10.00	10.00	20.00	50.00
<i>B. subtilis</i>	10.00	5.00	10.00	20.00	25.00
<i>E. coli</i>	10.00	5.00	10.00	20.00	25.00
<i>S. typhi</i>	20.00	10.00	10.00	20.00	25.00
<i>S. enteritidis</i>	20.00	10.00	20.00	20.00	50.00
<i>P. aeruginosa</i>	10.00	10.00	10.00	20.00	50.00
<i>K. pneumonia</i>	10.00	5.00	10.00	20.00	50.00
<i>C. albicans</i>	20.00	10.00	20.00	20.00	50.00
<i>C. krusei</i>	20.00	10.00	20.00	20.00	50.00

**Spectral Result**

The FTIR showed bands at 3409.3; 2924.18; 1461.13 and 1033  $\text{cm}^{-1}$  while  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400MHz):  $\delta$  3.2 (3H), 0.7 (5H), 5.2 (12H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100MHz):  $\delta$  143.5(C-13), 122.7 (C-12), 79.0 (C-3), 55.2 (C-5&18), 47.6 (C-9), 41.7 (C-14&22), 41.1

(C-8), 39.3 (C-19&20), 38.7 (C-1), 38.4 (C-4), 37.1 (C-10), 33.8 (C-17), 32.4 (C-7), 30.9 (C-21), 29.3 (C-2), 28.1 (C-28), 27.7 (C-23), 27.2 (C-15), 25.9 (C-16), 23.5 (C-11), 23.4 (C-27), 22.6 (C-30), 18.3 (C-6), 17.0 (C-29), 15.5 (C-26), 15.3 (C-24) and 14.0 (C-25).



**Fig- 1:  $^1\text{H}$ NMR of ABA**



## DISCUSSION

The antimicrobial activities of plant extracts have been linked to the presence of some bioactive compounds called secondary metabolites. These secondary metabolites also serve to protect the plants themselves against bacterial, fungal and viral infections [17]. These bioactive compounds are known to work synergistically to produce various effects on the human and animal subjects [18].

The screening of crude extracts of *Acacia ataxacantha* for phytochemical constituents showed that it contains flavonoids, steroids/ triterpenes, glycosides, tannins, saponins and alkaloids. Steroids are important drugs used as cardiac depressants, hypotensive, sedatives and anti-dysenteric agents [19]. Tannins have been reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects [20], and are used in ayurveda for the treatment of diseases like leucorrhoea, rhinorrhoea and diarrhea [2]. Alkaloids act as anti-malarial, anti-amoebic agents and astringents [19], while saponins exert a wide range of pharmacological activities including expectorant, anti-inflammatory, vasoprotective, hypocholesterolemic, immunomodulatory, hypoglycaemic, molluscicidal, antifungal, antiparasitic, hyperglycaemia, anti-oxidant, anti-cancer, weight loss and serve as natural antibiotics [21]. Flavonoids and tannins have been reported to possess antimicrobial activity; the antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall [22].

The antimicrobial screening of the crude extracts of the root of *Acacia ataxacantha* was carried out using 15 pathogens (*Bacillus subtilis*, *Corynebacterium ulcerans*, *Streptococcus pneumonia*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, *Candida tropicalis* and *Candida krusei*). The results showed that the test organisms *Bacillus subtilis*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans* and *Candida krusei* were sensitive to all the extracts while *Corynebacterium ulcerans*, *Streptococcus faecalis*, *Proteus mirabilis* and *Candida tropicalis* were resistant. This indicates that the extracts of *Acacia ataxacantha* has broad-spectrum of activity i.e against both gram positive, gram negative bacteria and fungi thus paved way for subsequent antimicrobial tests such as Zones of Inhibitions (ZI), Minimum Inhibition Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC).

All the crude extracts and ABA had significant ZI against all the test microorganisms except *Corynebacterium ulcerans*, *Streptococcus faecalis*, *Proteus mirabilis* and *Candida tropicalis*. EAF had the highest inhibition zone amongst the crude extracts, ranging from 20-30 mm, followed by CF with 20-25 mm, and then ME 20-24 mm and PEF with the least inhibition zone of 17-19 mm. ABA showed a higher activity (as compared to the crude extracts) with inhibition zone ranging from 24-31 mm (Table 2). This compares favourably with that of standard antibiotic drugs i.e Sparfloxacin, Cefuroxime and Fluconazole.

Minimum Inhibition Concentration (MIC) was done to determine the minimum concentration of the crude extracts and ABA that inhibited the growth of the microbes. It can be helpful in establishing the level of resistance of a particular bacterial strain and can substantially affect the decision to use certain antimicrobial agents. *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Salmonella enteritidis*, *Klebsiella pneumonia*, *Candida albicans* and *Candida krusei* were affected by 25 µg/mL of ABA while *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* were affected by 12.5 µg/mL concentration. PEF had MIC of 10 mg/mL; ME and CF had 5mg/mL for all the test organisms, while EAF had MIC of 5mg/mL for *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida krusei*; and 2.5mg/mL for *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonia* (Table 3) indicating that it is the most active (i.e. ethyl acetate) among the other extracts.

Determination of MBC/MFC was done to check whether the test microbes were actually killed by the extracts or only their growth was inhibited. The results indicated that petroleum ether extract had MBC/MFC of 20 mg/mL for all the test microbes; methanol and chloroform extracts had 10 mg/mL for *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* while ethyl acetate had 5 mg/mL for *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*. MBC/MFC results of ABA indicated that *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Salmonella enteritidis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida krusei* were killed at concentration of 50 µg/mL, while *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* were killed at concentration of 25 µg/mL (Table 4). These bacteria have been known to cause typhoid fever, muscle pains, diarrhoea with abdominal pains (*S. typhi*); hair follicle infection, food poisoning, pimples (*S. aureus*); meningitis, eye infection, infection of wounds and burns (*P. aeruginosa*) and urinary tract infection (*E. coli*), pneumonia, thrombophlebitis, cholecystitis,

upper respiratory tract infection, wound infection, osteomyelitis, meningitis, bacteremia and septicemia (*K. pneumonia*), bronchitis/rhinitis, acute sinusitis, otitis media and conjunctivitis (*S. pneumonia*) [23]. The result of MBC/MFC proves that *Acacia ataxacantha* can find its use in therapeutic preparations, especially in the diseases caused by *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonia*. Therefore, it can be said that the extracts of the root of *Acacia ataxacantha* can be used to treat infections like pneumonia, cough, dysentery and respiratory diseases (caused by these microbes) as practiced by traditional healers.

The compound isolated ( $\alpha$ -amyrenol) was reported to have anti-inflammatory, antinociceptive, gastroprotective and hepatoprotective properties and acts as an antioxidant [24] and is of medicinal importance in the treatment of liver disorders [25].

### Spectral Analysis

The Infrared spectroscopy of ABA showed absorption bands at 3409.3 that is characteristic of O-H stretching; 2924.18 for C-H stretching; 1461.13 for C=C stretching and 1033 for C-O stretching (Figure 4) [26], [27]. The  $^1\text{H}$  NMR spectra (Figure 1) showed peak at about 5.2 ppm corresponding to the methine proton of C-12; peak at about 3.2 ppm for the oxymethine  $^1\text{H}$  of C-3; 0.7 ppm for  $^1\text{H}$  of C-5. Peaks between 0.7-1.0 ppm revealed presence of methyl protons. The  $^{13}\text{C}$  NMR spectra revealed the presence of thirty (30) carbon atoms (Figure 2). The chemical shift at  $\delta$  79.0 signalled the presence of an oxy-methine carbon (C<sub>3</sub>). Signals at  $\delta$  122.7 and  $\delta$  143.3 represented olefinic carbons (C<sub>12</sub> and C<sub>13</sub>). Methylene groups (CH<sub>2</sub>) are responsible for signals observed at  $\delta$  38.7, 29.3, 18.3, 32.4, 23.5, 27.2, 25.9, 30.9 and 41.7. Distortionless Enhancement by Polarization Transfer (DEPT) experiment differentiates between CH, CH<sub>2</sub> and CH<sub>3</sub> groups (Figure 3). The spectra at 135° angle gave fifteen (15) peaks for CH and CH<sub>3</sub> in a phase opposite to nine (9) peaks for CH<sub>2</sub>. Spectra at 90° angle gave seven (7) peaks for CH groups, while 45° angle gave all carbons with attached protons (regardless of number) in phase. Signals from quaternary carbons (C) are always absent due to the lack of attached protons thus are identified as the additional signals in the proton decoupled  $^{13}\text{C}$  NMR spectra. The analysis thus indicates eight (8) CH<sub>3</sub> groups and six (6) C groups.

All  $^1\text{H}$  and  $^{13}\text{C}$  NMR data signals were found to be in full agreement with those reported for  $\alpha$ -amyrenol [28], [29], [30]. A pentacyclic triterpenoid (ursane) which contains a double bond between positions 12 and 13 and in which the hydrogen at the 3 $\beta$  position is substituted by a hydroxy group. The structure is given in Figure 5.

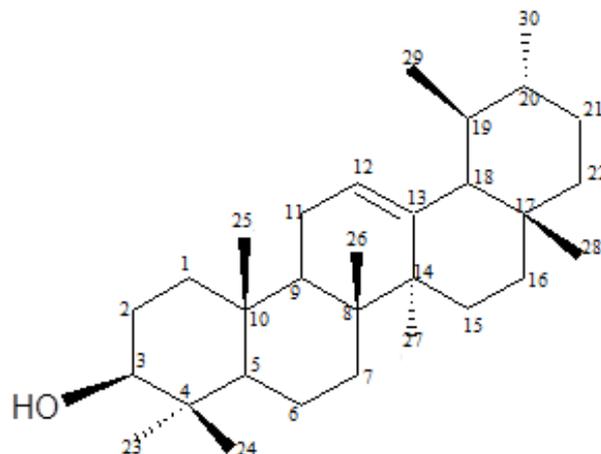


Fig- 5:  $\alpha$ -amyrenol C<sub>30</sub>H<sub>50</sub>O

**IUPAC Name** : (3 $\beta$ )-Urs-12-en-3-ol or 12-Ursen-3 $\beta$ -ol or Urs-12-en-3 $\beta$ -ol

**Other Names**: Viminalol,  $\alpha$ -amyrine,  $\alpha$ -amyrin

### CONCLUSION

The phytochemical analysis of the root bark of *Acacia ataxacantha* was found to contain some components which are of medicinal value. They include alkaloids, glycosides, flavonoids, tannins and steroids/triterpenes. Antimicrobial screening of root extracts from the plant showed that the extracts were able to inhibit the growth of some bacteria and fungi such as *Bacillus subtilis*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhi* and *Escherichia coli*; *Candida albicans* and *Candida krusei*. In conclusion, the findings in this research have justified the use of this plant in ethnomedical treatment of pneumonia, excessive cough, yellow fever, respiratory diseases, dysentery, and wounds which are caused by some of the organisms used in this study and other infections in which sparfloxacin and cefuroxime are used for treatment.

### REFERENCES

1. Amritpal SS; Herbalism, Phytochemistry and Ethnopharmacology. Science Publishers, Enfield, NH 03748, USA. CRC Press Taylor & Francis Group an informa business www.crcpress.com, 2011: 2, 40, 170, 178-185.
2. Obute GC, Osuji LC; Environmental Awareness and Dividends: A Scientific Discourse. African Journal of Interdisciplinary Studies, 2002; 3(1): 90 – 94
3. Ndukwe IG, Amupitan JO, Isah Y, Adegoke KS; Phytochemical and Antimicrobial Screening of the Crude Extracts from the Roots, Stem-bark and Leaves of *Vitellariaparatoxa* (GAERTN). African Journal of Biotechnology, 2007; 6 (16): 1905 – 1909.

4. Angiosperm Phylogeny Group (APG). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*, 2009;161 (2): 105–121. Retrieved 4 February, 2014.
5. Burkill HM; *The Useful Plants of West Tropical Africa*.1985;3:178-179.
6. Cheikhoussef A, Shapi M, Matengu K, Ashekele HM; Ethnobotanical study of indigenous knowledge on medicinal plant use by traditional healers in Oshikoto region, Namibia. *Journal of Ethnobiology and Ethnomedicine*, 2011; 7:10
7. Hedimbi M, Chinsebu KC; Ethnomedicinal study of plants used to manage HIV/AIDS-related disease conditions in the Ohangwena region, Namibia. *International Journal of Medical Plant Research*, 2012; 1(1):4-11
8. Oladunmoye MK, Kehinde FY; Ethnobotanical Survey of Medicinal Plants used in treating Viral Infections among Yoruba Tribe of South Western Nigeria. *African Journal of Microbiology Research*, 2011; 5(19): 2991-3004.
9. Dambatta SH, Aliyu BS; A Survey of Major Ethno Medicinal Plants of Kano North, Nigeria, their Knowledge and Uses by Traditional Healers. *Bayero Journal of Pure and Applied Sciences*, 2011; 4(2): 28 – 34
10. Kadiri AB, Olowokudejo JD, Travih VA; An Ethnobotanical Survey of Herbal Markets and Medicinal Plants in Lagos State of Nigeria. *Ethnobotanical Leaflets*, 2008; 12: 851-65.
11. Sofowora EA; *Medicinal Plants and Traditional Medicine in Africa*. 2<sup>nd</sup> ed. Spectrum Books limited Ibadan, Nigeria 1993; 9-25, 289
12. Trease GE, Evans WC; *A Textbook of Pharmacognosy*, 13<sup>th</sup> ed. Bailliere Tindall Ltd, London, 1989; 134
13. Bauer AW, Kirby WM, Sherris JC, Turck MC; Antibiotic Susceptibility Testing by A Standardized Single Disc Method. *American Journal of Clinical Pathology*, 1966; 45:493-496
14. Barry AL, Thornsberry C; Susceptibility Tests: Diffusion Procedure. *Journal of Clinical Pathology*, 1985; 19: 492.
15. Magaldi S, Mata-Essayag S, Hartung C, Perez C, Colella M.T, Olaizola C et al., Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*, 2004; 8 (1): 39-45.
16. Thornsberry C, Donald RS; Broth-Dilution Method for Determining the Antibiotic Susceptibility of Anaerobic Bacteria. *Antimicrobial Agents and Chemotherapy*, 1975; 7(1):15-21
17. El-MahmoodAM, AmeyJM; In Vitro Antibacterial Activity of *ParkiaBiglobosa* (JACQ) Root Bark Extract against some Microorganisms Associated with Urinary Infections. *African Journal of Biotechnology*, 2007; 6 (11): 1272-1275
18. Amagase H; Clarifying the Real Bioactive Constituents of Garlic. *Journal of Nutrition*, 2006; 136:716-725
19. Ghani A; *Introduction to Pharmacognosy*.1<sup>st</sup> Ed. ABU Press Ltd Zaria- Nigeria. 1990; 187-197
20. Trease GE, Evans WC; *Pharmacognosy*. 15<sup>th</sup> ed. Elsevierpublishers Ltd. Edinburgh London, 2002; 20-23
21. Podolak I, Galanty A, Sobolewska D; Saponins as Cytotoxic Agents: A Review. *Phytochemistry Reviews*, 2010; 9(3): 425–474.
22. Cowan MM; *Plant Products as Antimicrobial Agents*. *Clinical Microbiology Reviews*, 1999; 12(4): 564-582.
23. Greenwood D, Richard BS, John FP; *Medical Microbiology: A guide to Microbial Infections, Pathogenesis, Immunity, Laboratory Diagnosis and Control*. Longman Group Ltd. Hong Kong, 1992; p.211
24. Melo CM, Bezerra MKC, Neves CSJ, Morais TC, RaoSV, Santos FA et al.,  $\alpha,\beta$ -amyrin, A Natural Triterpenoid Ameliorates L-Arginine-Induced Acute Pancreatitis in Rats. *World Journal of Gastroenterol*, 2010; 16(34): 4272–4280.
25. Oliveira FA, Chaves MH, Almeida FR, Lima, RC Jr, Silva RM, Maia JL et al., Protective effect of alpha- and beta-amyrin, a triterpene mixture from *protiumheptaphyllum* (aubl.) March.Trunk wood resin, against acetaminophen-induced liver injury in mice. *Journal of Ethnopharmacology*,2005; 98(1-2):103-8.
26. Coates J; Interpretation of Infrared Spectra. A Practical Approach: In *Encyclopedia of Analytical Chemistry* R.A. Meyers (Ed.), John Wiley & Sons Ltd, Chichester. 2000; 10815–10837.
27. David JM, Silva ML, DavidJP, Silva LC, Santos RAF, Lima LS et al., Bioactive oleanane, lupane and ursanetriterpene acid derivatives. *Molecules*, 2012; 17: 12197-12205
28. Vazquez HL, Javier P, Arturo N; The PentacyclicTriterpenes  $\alpha$ ,  $\beta$ -amyryns: A Review of Sources and Biological Activities, *Phytochemicals - A Global Perspective of their Role in Nutrition and Health*. Available from <http://www.intechopen.com/books/phytochemicals-a-global-perspective-of-their-role-in-nutrition-and-health/the-pentacyclic-triterpenes-amyryns-a-review-of-sources-and-biological-activities> 2012.
29. Niaz A; Brine Shrimp Citotoxicity of crude methanol extract and Antispasmodic Activity of  $\alpha$ -amyryn acetate from *Tylophora hirsute* wall. *BMC Complementary and Alternative Medicine*, 2013; 13:135
30. Fingolo CE, Santos TS, Marcelo DMV, Kaplan CMA; Triterpene Esters: Natural Products from *Dorstenia arifolia* (Moraceae). *Molecules*, 2013; (18): 4247-4256.