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ANTIMICROBIAL ACTIVITY OF STIGMAST-4-EN-3-ONE AND 2,4-DIMETHYLHEXANE ISOLATED FROM *NAUCLEA LATIFOLIA*.

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ABSTRACT

The methanol extracts of the stem bark and leaf of Nauclea latifolia (Rubiaceae) were investigated for their phytochemical constituents and antimicrobial activity. The result revealed that the plant contain bioactive constituents such as flavonoids, tannins, alkaloids, terpenes, and steroids. Two compounds were isolated from the plant, a steroid and an alkane. The steroid, stigmast-4-en-3-one was isolated from the ethylacetate fraction of the stem bark and the alkane, 2,4-dimethylhexane from the n-hexane fraction of the leaf. The stem bark extract, the leaf extract and the two compounds isolated exhibited antimicrobial activity against some bacteria including *Streptococcus gordonii* and *Streptococcus sanguinis*. Both of them are oral bacteria believed to be responsible for dental caries.

Keywords: Antimicrobial, Stigmast-4-en-3-one, Isolation, 2,4-dimethylhexane.

INTRODUCTION

Nauclea latifolia (Rubiaceae) is a multi-stemmed tree with an open canopy. It is found in Guinea, Mali, Togo, Uganda, Cameroon, Gambia, Ghana and Nigeria [1]. Previous studies show that the plant has entihypertensive, antiplasmodial, antimalarial, antiviral and antipyretic activities [2-5].

MATERIALS AND METHODS

Extraction

The fresh stem bark and leaf (1.0kg each) of *Nauclea latifolia* were collected, shade dried, pulverized and macerated separately for 72 hours with 2.5 litres of methanol each. The extracts were evaporated. The concentrated extracts were partitioned into n-haxane, and ethylacetate fractions.

The dried ethylacetate extract (6.3g) was used to run an open column Chromatography. About 40g of silica gel (particle size 60-120) was poured in 120ml of ethyl acetate and stirred. The slurry formed was packed in the column of dimension 3 x 45cm.

The dried ethylacetate extract (6.3g) mixed with about 5.0g of silica gel was crushed into powder. The powder was loaded on the slurry and the column was eluted using ethylacetate alone followed by ethlacetate-methanol combination, then methanol alone and finally methanol-water combination. The eluents collected were subjected to thin layer chromatography (TLC) and preparative TLC . The TLC of one the bands gave one yellow spot when sprayed with 10% H_2So_4. The R_f of the spot was 0.56 and the compound was designated compound **A**

Spectroscopic Analysis

The chromatogram was obtained using the Gas chromatography hyphenated with mass spectrometer (GC-MS), model QP 2010 plus Shimadzu, Japan. The experiment was performed at a column oven temperature of 60^{o}C and injection temperature of 250^{o}C at a pressure of 100.2 kpa with a total flow rate of 6.2ml per minute.

RESULTS AND DISCUSSION

The chromatogram (mass spectroscopy) of compound **A** was run at a retention time of 34.2 seconds. The base peak of 124 was obtained by loss of 288. The compound gave a molecular weight of 412, molecular formula of $C_{29}H_{48}O$ and the following major fragments 397 (M^+, 15%), 370 (20%), 288(35%), 299 (55%), 124 (100%), 57 (6 %) and 43 (95%).

Infrared Spectroscopy (IR) of Compound **A** was obtained on Fourier Transform infrared spectrophotometer, model FT-IR – 8400S, Shimadzu, Japan. The absorbance at 1029.06 is typical of C-H bend while 1449.55 is typical of a C-H scissoring. There was strong carbonyl (C=O stretch) absorbance at 1646.30. The absorbances at

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2832.56 and 2939.61 indicate SP3 C-H stretching.

The standard proton parameters (^{1}HNMR) of compound A was recorded in dimethyl sulfoxide (DMSO) for compound A and deuterated Chloroform ($CDCL_3$) for compound **B** on a Varian XL – 400 spectrophotometer operating at 400.13MH_Z at ambient temperature.

The same operator (Vnmr 1) was used for the standard carbon experiment (^{13}CNMR) using DMSO as the solvent and tetramethyl silane (TMS) as internal standard. The Infrared Spectroscopy (IR) was recorded on Fourier Transform infrared spectrophotometer, model 8400S, Shimadzu, Japan.

The following chemical shifts (δ) were obtained for ^{1}NMR of Compound **A**: 0.95:3H of 29-CH_3, 2.0:3H of 19-CH_3, 3.5:2H of side chain, 5.5:olefinic H in CH_2, 7.3 and 11.0:1H each of backbone.

The total number of carbon signals in the spectrum of^{13}CNMR of Compound **A** was 29. This included one carbon signal of carbonyl group at 171.0 and one carbon signal of olefinic carbon at 123.0.The presence 29 carbon signals, one carbonyl carbon, one olefinic carbon and six isolated CH_3 at C-18 (11.2), C-19 (17.7), C-21 (17.5), C-26 (19.0), C-27 (18.3) and C-29(11.5) suggest that compound **A** is a steroid.

Stigmast-4-en-3-one was previously isolated from spillanthes *acemella* murr. (compositae). The plant is used traditionally in India to treat tooth ache [6]. This justifies the traditional use of the stem of *Nauclea latifolia* as chewing stick in Nigeria [5, 7].

The powdered dried leaf of *Nauclea Lafifolia* (1.0kg) was macerated with 2.5 litres of distilled water at room temperature for 72 hours and filtered. The filtrate was partitioned to obtain in n-hexane extract which was evaporated to dryness with rotary evaporator.

The concentrated n-hexane extract (3.8g) was used to run an open column chromatography. About 50g of silica gel (particle size 60-120) was dissolved in 120ml of n-hexane and stirred. The slurry formed was packed in the column (3 x 45cm). The concentrated n-hexane extract (3.8g) was mixed with about 5g of silica gel and crushed into powder. The powder was loaded on the slurry and column eluted. The eluents obtained were monitored by TLC and preparative TLC. The TLC (100% n-hexane) gave colourless oily liquid with one spot under UV shortwave (254nm). The R_f value of the spot was 0.64.

The retention time in the GC-MS of Compound **B** was 4.1 seconds. The molecular weight of the compound was 114 and the molecular formula C_8H_{18}. The base peak of 43 (100%) was obtained by loss of 91. The major fragments were 114 (2%), 99 (1%), 85 (48%) 71 (15%), 57 (70%) and 43 (100%). The spectral library confirmed the name of the compound to the 2,4-dimethylhexane.The spectra generally consist of clusters of peaks separated by fourteen mass units (CH_2) at M/Z 29, 43, 57… (CnH_2n^{+1}). The ions are one or two mass units lower due to loss of hydrogen e.g. (27, 28, 29), (41, 42, 43), and the intensity increases with decrease in M/Z to a maximum at 43 or 57 [8].

The FT-IR of Compound B gave the following absorptions:

1383.97:C-H Methyl rock for alkane, 1461.13:C-H Scissoring for alkane, 2867.28:C-H Stretch for alkane, and 2942.51: C-H Stretch for alkane. The splitting pattern in the ^{1}HNMR of Compound **B** showed mainly a cluster of various doublets and triplets situated between 0.95 and 1.58. signals (δ) occurred at: 1.28, 0.96, 1.58, 0.86, 0.82 and 0.84.

The spectroscopic data obtained in this study are in accordance with already reported data for 2,4-dimethylhexane. The refractive index of the compound was 1.41 and the boiling point ranged from 107-110^{o}C compared to the literature values of 1.39 and 108-109^{o}C respectively [9].

In a study 2,4-dimethylhexane was previously isolated from *Tragia involucrate* (Euphobiaceae). The isolated compound decreased oedema induced by snake venom [10].

This study seems to suggest that the wound healing activity of *Nauclea Latifolia* earlier reported is due to the presence of 2,4-dimethylhexane in the plant [11].

Antimicrobial Activity Study

Antimicrobial activity was measured using Agar Well Diffusion Assay; [12]. Mueller-Hinton agar medium was used for anti bacterial analysis at a PH of 7.4 and Sabouraud dextrose agar for antifungal analysis. Eight (8) bacterial and two (2) fungal species were investigated. Phytochemical screening was carried out using the standard method [13].

The concentrations are 100mg/ml (stem and leaf extracts), 50µg/ml (ciprofloxacin, compounds **A** and **B**) and 54µg/ml (Nystatin). *Nauclea Latifolia* show antimicrobial activity in the crude methanol extract. All the bacteria including *streptococcus gordonii* and *streptococcus sanguinis* (oral bacteria that cause dental caries) were inhibited.

The steroid stigmast-4-en-3-one isolated from the stem and the alkane 2,4-dimethylhexane from the leaf show antimicrobial activity. The extracts and the compounds isolated did not show antifungal activity. The phytochemical screening show that *Nauclea Latifolia* contains bioactive constituents such as flavonoids, tannins alkaloids and terpenes.

Table 1. ^{13}CNMR of Compound A

Carbon atom	(PPM)
C-1	35.0
C-2	33.5
C-3	171.0

C-4	123.0
C-5	199.0
C-6	32.7
C-7	32.4
C-8	35.0
C-9	53.0
C-10	38.0
C-11	21.8
C-12	39.0
C-13	42.0
C-14	55.0
C-15	24.8
C-16	27.9
C-17	55.1
C-18	11.2
C-19	17.7
C-20	35.9
C-21	17.5
C-22	32.3
C-23	24.4
C-24	45.0
C-25	28.2
C-26	19.0
C-27	18.3
C-28	23.1
C-29	11.5

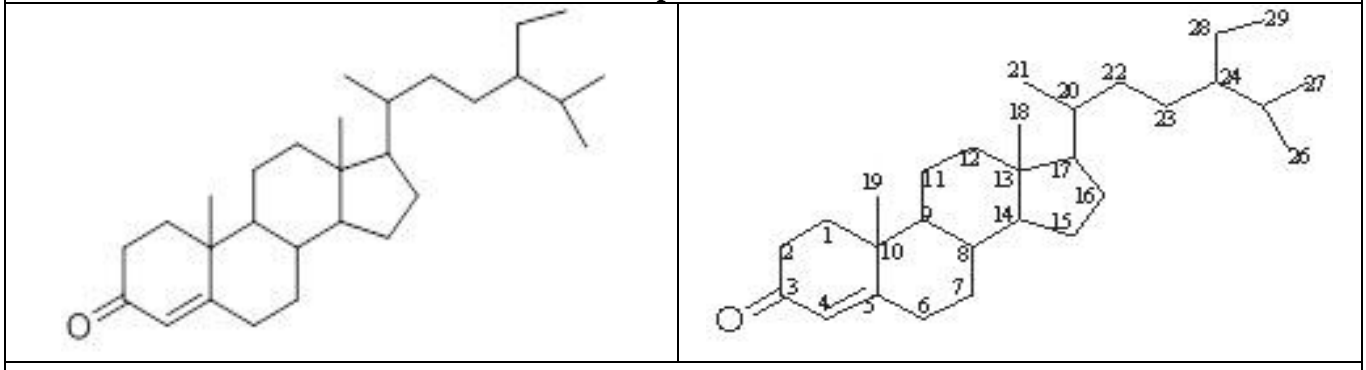
Table 2. ^{13}CNMR of Compound B

Carbon No	Chemical Shift (PPM)
C-1	24.979
C-2	25.301
C-3	32.666
C-4	32.358
C-5	28.010
C-6	9.399
C-7	22.666
C-8	22.358
C-9	24.979

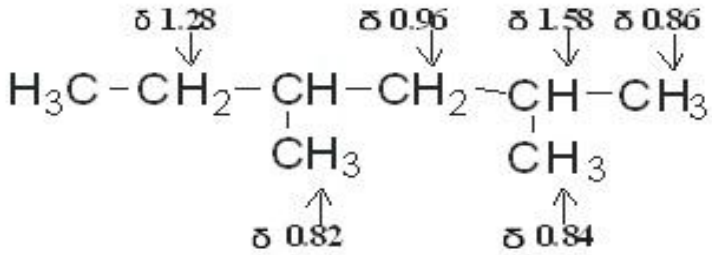
Table 3. Inhibition Zone Diameter (Mm) Of Extracts And Compounds From Nauclea Latifolia

	Test organism	Stem	Leaf	Comp. A	Comp. B	Cypro	Nyst.
1	*Bacillus subtilis* NCTC 8853	14	12	8	7	27	-
2	*Escherichia coli* ATCC 25922	8	6	5	4	29	-
3	*Pseudomonas aeruginosa* ATCC 15442	10	8	6	5	26	-
4	*Staphylococcus areus* NCTC 571	9	7	5	4	25	-
5	*Streptococcus gordiniii*	13	10	7	6	23	-
6	*Streptococcus sanguinis*	12	9	7	5	25	-
7	*Candida albicans*	-	-	-	-	-	12
8	*Salmonella typhii*	10	8	6	5	27	-
9	*Shigella*	14	12	8	7	24	-
10	*Trichophyton tonsurans*	-	-	-	-	-	10

Compound A



Compound B



2,4-Dimethylhexane

CONCLUSION

The stem bark of *Nauclea Latifolia* contain the steroid stigmast -4-en-3-one while the leaf contain an alkane 2,4-dimethylhexane. The two compounds isolated were active against *streptococcus gordonii* and *streptococcus sanguinis* (oral bacteria responsible for dental caries).

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